

Control Release from Biopolymers

by

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Abstract

Control release from biopolymer is an important issue for flavour perception following to reduce flavour content such as salt in food formulation without any tangible change in taste. Having reviewed the literature, a relevant biopolymer has been chosen which enable investigation into the mechanical properties and microstructure of the biopolymer, then, flavour release from the biopolymer.

The experimental investigation presented here examines release behaviour from low acyl gellan gum gel. Attempts have been initially focused on the investigation of the mechanical properties, and subsequently to find a link between mechanical properties and microstructure of the gel. This study is based on the relationship between mechanical properties and the microstructure of the gellan gel. Based upon obtained results which present investigation in relation to the mechanical properties during the compression. After conducting the compression tests, a number of parameters were investigated and the consist of the effect of the gellan concentration, salt concentration and cyclic compression on the mechanical properties of the gel. According to the results, mechanical properties of the gellan gels were remarkably affected by the gellan and salt concentration. In addition, following the cyclic compression the mechanical properties of the gellan gels were significantly affected.

It was shown that the gellan and salt concentration play a main role on the microstructure of the gel. Also, the microstructure of the gel can be significantly affected by the cyclic compression.

The release experiments were carried out using uniaxial and cyclic compression to investigate the salt and riboflavin release from the gellan gel to identify the parameters which play a role on release from the gellan gel. Release experiments have shown that release profile is affected by the gellan and salt concentration, also, number of cyclic compression and compression mode play a major role on salt and riboflavin release. Results demonstrated that release profile can be affected by molecular weight of the releasable material.

Dedication

This thesis is dedicated to the memory of my dear father. I miss him every day, but I am glad to know he saw this process through to its completion, offering the support to make it possible, as well as plenty of friendly encouragement.

I would also like to dedicate this thesis to my wife, Maryam and my beloved daughter Sarina. Their love, patience and understanding have lightened up my spirit to finish this study and this thesis.

Last but certainly not least, I would like to dedicate this thesis to my mother who has always supported me whatever I have done.

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List of Abbreviations

CEM	-	Cryo Electron Microscopy
CRT	-	Cathode Ray Tube
Cryo-SEM	-	Cryo Scanning Electron Microscopy
DNA	-	Deoxyribo Nucleic Acid
ECM	-	Extra Cellular Matrix
FDA	-	Food and Drug Administration
FESEM	-	Field Emission Scanning Electron Microscope
KGM	-	Konjac Gluco Mannan
RTE	-	Ready-To-Eat
SEM	-	Scanning Electron Microscopy
TPA	-	Texture Profile Analysis
TPD	-	Texture Profile Analyzer
USA	-	United States of America
VTD	-	Vacuum Transfer Device
WHC	-	Water Holding Capacity
WHO	-	World Health Organization

List of Symbols

A_0	-	Original cross section area
F_t	-	Normal force at time (t)
H_0A_0	-	Initial cross sectional area and height of the sample
H_t	-	Height of the sample at time (t)
A_c	-	Permeable area
E_d	-	Plastic deformation Energy
E_f	-	Friction Energy
E_r	-	Energy for Release
$F_{(t)}$	-	Force at time t
L_0	-	Original Length
$W_{d,c}$	-	Energy spent as a result of Friction
$W_{d,v}$	-	Energy consumed because of Visco-Elasticity
W_e	-	Strain Energy
W_f	-	Energy used to Fracture
$\frac{dh}{h}$	-	Length Of The Sample
h_0	-	Original Height of sample
ϵ_H	-	Hencky Strain
ϵ_b	-	Strain at break

List of Symbols

σ_b	-	Breaking Stress
ΔL	-	Change in Length
Δp	-	Pressure difference
A	-	Cross section area
F	-	Force
F_m	-	Maximum Force
r	-	Radius Of The Sample
B	-	Permeability coefficient
E	-	Total Energy
E	-	Young's modulus
G	-	Storage Modulus
L	-	Length
Q	-	Volume flow rate of serum
W	-	Energy supplied to the sample
e	-	Cauchy Strain
h	-	Height of sample
l	-	Distance
ε	-	Strain
η	-	Viscosity of liquid
η	-	Viscosity of the Liquid
σ	-	Stress

Chapter 1. Introduction

1.1. Overview

The purpose of this thesis is to understand the control release from biopolymers. This aspect is an essential issue in the food industry as these biopolymers release usually are in the form of salt and sugar flavour. Sodium plays a vital role in the health of an individual which is why salt is considered as a necessary substance. Excessive consumption of these components could lead to severe illness and heart disease and majority of the individuals consume without being aware of these facts. The taste and structure of the food products are affected to a large extent by the level of salt usage, which is why it is considered as one of the most important flavours for the food industry. It also has the ability to decrease the water activity and increase the microbiological stability. Hence, the food industry struggles to reduce the salt content present in processed food. The only appropriate way to reduce the salt content would be to manage it without carrying out a tangible change in the saltiness.

Hence, it is considered to be necessary to establish a technique which would reduce the level of salt intake in individuals and prevent all illness and disadvantages associated with it. There should be no tangible change in the saltiness while reducing the salt content during the food formulation.

The taste and structure of the products are affected to a large extent by salt which is why it is considered as one of the most important flavours for the food industry. It also has the ability to decrease the water activity and increase the microbiological stability. Hence, the food industry struggles to reduce the salt content present in

processed food. The only appropriate way to reduce the salt content would be to manage it without carrying out a tangible change in the saltiness.

Biopolymers consist of functional properties that can be used for formulated foods, which is why these are considered as essential food ingredients. It helps as a texture stability developer as well as acts as an agent for gelling or thickening. The usage of biopolymers has increased as it helps the food manufacturers in reducing the salt content in the food products. Due to the increase in demand for salt reduced processed food products the research on reducing the salt content in food products has become the highly profound. The consumers increasingly require the reduction of salt content in their foods specifically processed foods which is why the use of this ingredient has become essential. In addition, the manufacturers must be well aware of the technical and mechanical properties which using the biopolymers. Foods which have biopolymers in their formulation have a relation with the mechanical properties and their microstructure. This relationship is considered to be significant as it is responsible for the flavour release. Moreover, it is essential to analyze the mechanical properties and microstructure of the gels as it identifies the behaviour of the gels during compression which is followed by the release of flavour.

The key objective of the study is to change the perception of the consumers by analyzing the flavour mechanism. For this purpose, low acyl gellan gum gels which release salt were prepared and compression tests were carried out to analyze the behaviour. Water, fracture, salt release and microstructure are related with the mechanical properties. Mastication mechanism was to be mimicked, its effect on mechanical properties of the gels and flavour release was to be checked which is

why Cyclic compression tests were carried out. Hence, with the different salt release profiles the study provides the design of salt matrices.

The Cryo Scanning electron microscopy (Cryo-SEM) is used to capture images of the microstructure of the gels which links the microstructure of the gels and mechanical properties, effect of the gellan and salt concentration. Thus, this process is used during the cyclic compression on the microstructure of the gels and the above mentioned parameters are considered while analyzing the images through appropriate software.

The effect of molecular weight and size of flavour upon the gel release rate need to be analyzed which is why different flavours of molecular weight and size have been taken into account.

1.2. Overview of Thesis

Chapter 1 discusses about the study introduction, aim and objectives and the thesis's overview. Mechanical properties, microstructure and flavour release are reviewed in Chapter 2. The chapter also consist of mechanical properties definition, low acyl gellan gum structure and food industry application, gel definition, microstructure of the gel, effect of salt and sugar on mechanical properties and a brief review of the Cryo Scanning Electron Microscopy's application in order to investigate the microstructure of hydrocolloid gels. Additionally, the review analyzes the flavour release from hydrocolloid gels and the microstructure of the gels and release correlation and especially covers the effect of salt and hydrocolloid concentration on flavour release.

In Chapter 3, describes the material and methods used in the study. The section also states the uniaxial compression, riboflavin release, cyclic compression, salt release and experimental equipment and methods description. The statistical techniques used in the analysis are described which includes the methods to measure salt and riboflavin release following compression and Cryo Scanning Electron Microscopy to investigate the microstructure of the gels.

In Chapter 4, the effect of cyclic compression on mechanical properties and the geometry of the gels are analyzed along with the effect of salt and low acyl gellan gum concentration on mechanical properties of the gel.

Chapter 5 investigates the microstructure of the gels through the Cryo Scanning Electron Microscopy used for the experiments. Various images of low acyl gellan gels with different salt and gellan concentrations were captured along with gels after cyclic compression to understand the effect of cyclic compression on microstructure of the specimens.

Chapter 6 reports the salt and riboflavin release from the low acyl gellan gum gels have been described by using Uniaxial and cyclic compression methods. This would help to identify the parameters that play a vital role in the gel release.

The key findings and developments have been summarized in Chapter 7 after analyzing the control release from biopolymers. The limitations, recommendations and future development have also been mentioned as part of this section.

Chapter 2. Literature Review

2.1. Introduction

In this chapter, a synopsis of the diverse scientific and engineering features of the research is presented. Also, the significance of the detailed research and the analyses conducted to support the research are included along with the empirical evidence in this chapter. In order to attain desirable microstructural relationships associated with the impact of alterations in gel microstructures on the release of flavour in the gellan gum gels, it is important to recognize the effect of production processes of hydrocolloid gel on their mechanical behaviour and the influence of this mechanical behaviour on low acyl gellan gum gels.

Section 2.2 presents the definition of the gel, while Section 2.3 elaborates the structure and characteristics of gellan gum. Moreover, Section 2.4 explicates the mechanical properties, Section 2.5 describes the effect of the additive materials on the mechanical properties of the gels and Section 2.6 describes the texture of the gels. Explanation regarding the flavour released by low acyl gellan gum is given in Section 2.7 and the Scanning Electron Microscopy Technique (SEM) is detailed in Section 2.8.

2.2. Gel Definition

Ferry (1980) defines gel as: *“a substantially diluted system which shows no steady state flow”* (Ferry 1980). From the above definition it applicable for the solid materials exhibiting the same characteristics of quantity of solvent can be categorized into the same category. Gel is basically a fusion of two constituents (i.e. gelling polymer and

solvent). The matrix comprises of a continuous arrangement of solid materials that contains a continuous or uniformly distributed liquid phase (Olsson et al. 2002). Due to their quality to effectively depict the mechanical properties of different food systems, gels have been applied as illustrative models to understand food textures; this makes them useful in acquiring information regarding food texture (Jones et al. 2002). Gellan gum is one of the most prevalent hydrocolloids that are utilized as a purposeful ingredient to impart the essential textural qualities in food products (Funami, 2011).

Food industries uses the gel-forming polymers, especially in processed food products, since the molecules of the polymer hold the ingredients of the processed foods in a three-dimensional structure and give it a solid-like shape by connecting at the points. Polymeric gel-formers can be readily used as substitutes of starches, proteins, fats and low molecular weight sugars to provide the required textures in sauces, emulsions and custards (Saha & Bhattacharya, 2010). This is due to the fact that the caloric values of some gel-forming polymers are very low or almost negligible as they are hard to digest.

The functional characteristics of hydrocolloids has extensive application in the processes involved in food industries, such as gelling, emulsifying, thickening, binding, suspending and coating (Burey *et al.*, 2008; Saha & Bhattacharya, 2010). Although hydrocolloid gels are not much used as a final product, they are mostly added in food products as additives to boost or manipulate the food's characteristics. The most attractive trait of hydrocolloid gels is their potentiality to produce aqueous solutions which are highly viscous at low concentrations of gel and has desirable uniformity (Williams, 2006). Also, they can be used to produce gels with various

strengths and constancy. In addition, they are usually applied as alternatives to some ingredients, especially in processed foods which has low fat and low calories (Tang et al. 1994).

2.3. Previous literature on Gels or Compounds

Hydrocolloids (Phillips & Williams, 2000), is also known as gums, which are hydrophilic polymers derived from vegetable, animal, microbial or synthetic origin. In general it contains many hydroxyl groups which might be polyelectrolytes. These hydrocolloids are available in the nature in order to maintain the functional property of the aqueous foodstuffs (Table 2.1). The major functional property is viscosity, which includes thickening, gelling and water binding (Torres *et al.*, 2012). However, other significant property includes emulsion stabilization, prevention of ice recrystallisation and organoleptic properties. The degree with which the hydrocolloid solutions mix with saliva determines the degree of chain entanglement and flavour perception (Ferry *et al.*, 2006). The major application of hydrocolloids includes adhesion, suspension, flocculation, foam stabilization and film formation. Foodstuffs are very complex materials and this together with the multi factorial functionality of the hydrocolloids has resulted in several different hydrocolloids being required.

Primarily, gums were used mainly as additives for food products (Totosa & Pérez-Chabela, 2009; Imeson, 2010; Banerjee & Bhattacharya, 2011). Simultaneously, natural gums or their derivatives have been broadly investigated as excipients for pharmaceutical or biomedical purposes (Dumitriu, 2002; Rehm, 2010). Matrix tablets (Rasool *et al.*, 2010; Vijan *et al.*, 2012), soft or cross-linked hydrogels (Shalviri *et al.*, 2010), floating beads (Verma & Pandit, 2011), pellets (Santos *et al.*, 2004),

microspheres (Sullad *et al.*, 2011; Kajjari *et al.*, 2012), in situ forming systems (Miyazaki *et al.*, 1999) or transdermal films (Mundargi *et al.*, 2007) are the common examples.

Various gelling agents have different applications for instance Agar derived from seaweeds, which is used as a luxurious vegetarian gelatin substitute, in jellies and Japanese desserts such as anmitsu (Armisen *et al.*, 2000; Stanley, 2006). Likewise Cereal flour and starch is used as a Secondary gelling agent, cost effective, rice flour based gels (Jena & Bhattacharya, 2003; Alka & Bhattacharya, 2008). Cellulose is derived from plant cell wall and used in salad dressings and desserts (Williams & Philips, 2000). Carrageenan is used in Desserts, gel to immobilize cells/ enzymes (Williams & Philips, 2000). The various application of Xanthan gum includes Salad dressings and sauces, helps to stabilize the colloidal oil and solid components against creaming by acting as an emulsifier and texture modifiers in different food (Sutherland, 2007; Doublier & Cuvelier, 2006).

Table 2.1 Gelling agents and its application.

Gelling agent	Source of hydrocolloids	Applications	Author
Pectin	Plant derivative (citrus peel, guava, apple)	Jam, jelly, marmalade, jujubes, yogurt	Williams and Philips (2000); Oakenfull (1987)
Agar	Red algae / seaweeds	Used as laxative, vegetarian gelatin substitute, in jellies and Japanese desserts such as anmitsu	Armisen <i>et al.</i> , (2000); Stanley (2006)
Gum Arabic	Plant derivative	Hard gummy candies, chocolate candies and chewing gums	Sutherland (2007)
Locust bean gum	Extracted from the seeds of the Carob tree	Gelling agent	Cui <i>et al.</i> (2007); Schorsch <i>et al.</i> (1997)
Guar gum	Endosperm of guar gum	Pastry fillings, yogurt, liquid cheese products and sweet desserts	Cui <i>et al.</i> (2007); Richardson <i>et al.</i> (1998)
Gum Karaya	Extract of Sterculia trees	Brown sauce, toppings, fillings	Le Cerf <i>et al.</i> (1990); Weiping and Branwell (2000)

Gelling agent	Source of hydrocolloids	Applications	Author
Gum Tragacanth	Dried sap of several species of legumes of the genus Astragalus	Confectionary icings, dressings	Aspinall and Bail Lie (1963)
Alginate	Brown seaweeds	Jellies, gelation with divalent cations, cell immobilization and encapsulation, appetite up present	Roopa and Bhattacharya (2008)
Gelatin	Animal skin and bones	Gelling agent in gelatin desserts, jelly, trifles and confectioneries, jam, yogurt, cream cheese and margarine	Ward and Courts (1977); Schrieber and Gareis (2007); Oakenfull (1987)

One of the most extensively studied and described member of bacterial polysaccharides is gellan gum which is commercialized in USA and Japan (Morris *et al.*, 2012). In pharmaceutical industry gellan gum has been used in oral drug delivery, mainly as a disintegrating agent in immediate release tablets (Shiyani *et al.*, 2009) or a matrix-forming excipient for sustained release (Vijan *et al.*, 2012; Franklin-

Ude *et al.*, 2007). The previous reports which focused on the application of gellan gum in various areas of medicine is still emerging. Gellan has been considered as a material for preparation of dental cavity fillings after tooth extraction (Chang *et al.*, 2012). Gellan gels advantageous properties such as rapid gelation in the presence of cations, biodegradability, muco adhesive potential or high water holding capacity, non-toxicity. These properties make gellan gel as a useful component in various industries such as biomedical, pharmaceuticals, food industries, and multiple oral, ophthalmic, nasal and other formulations. Due to a large variety of potential applications of gellan gum, several reviews have been prepared (Morris *et al.*, 2012; Prajapati *et al.*, 2013). Application of gellan in food industry is increasing due to its functional properties. Gellan is used for production of liquid gels, edible films, gelatin desserts, jams and microencapsules. The following section briefly explains the gellan gum.

2.4. Gellan Gum

Kelco Division, Merck and Co gave the trade name of 'gellan gum' to a deacetylated extracellular polysaccharide from the bacteria *Pseudomonas elodea* (Nishinari, 1999). The gellan gum has been a subject of interest since its discovery in 1980 due to its ability to form transparent gels even at low concentrations (Rodriguez-Hernandez *et al.*, 2003). In addition, traditional gelling agents such as agarose and carrageenan show reduced gelling capacity at low pH, whereas gellan gum can form strengthened gels (Moritaka *et al.*, 1995; Yamamoto & Cunha, 2007). Gellan gum's use in food products was approved by FDA in 1992.

2.4.1. Features of Gellan Gum

Gellan gum appears as an off-white powder and it is soluble in water. Its molecular weight ranges from 70,000 Daltons to 95 percent over 500,000 Daltons. The addition of positively charged ions (i.e., cations) activates its gel-forming ability. Its melting temperature can be varied to be either below or above 100 °C. Similarly, controlled incorporation of potassium, magnesium, calcium, and/or sodium salts can help direct the thickness and texture of gellan gum in different products (Mao et al. 2000).

Gellan gum is an additive used in food products for thickening or gelling, and can impart diverse gel-like textures in them, from rigid plus brittle to liquid-like. Some processed products that are usually added with gellan gum are: dairy products, microwavable foods, bakery fillings, structured foods, sauces, frostings, confections, icings, dessert gels, jams, jellies, glazes, low-fat spreads, toppings and puddings (Saha & Bhattacharya, 2010). Gellan gum is the whitish powder having no specific flavor and odour. It can also crumble at 150 °C, without melting and soluble in cold water. Gel can be produced through gellan gum under following conditions:

- It must be heated prior to any other process;
- The gellan gum solution contains a definite amount of cations, which makes it form gel after the solution cools down;

The strength, formation temperature and the melting temperature of the gel depends upon the quantity and type of the cations present (Tang *et al.*, 1994, Lau *et al.*, 2000). Table 2.2 shows the characteristics and efficiency of low acyl gellan gum (Williams, 2006; Saha & Bhattacharya, 2010).

Table 2.2 Characteristics and efficiency of gellan gum. **Source: Adapted from Williams, 2006; Saha & Bhattacharya, 2010**

<i>Characteristics</i>	<i>Efficiency</i>
Produces very competent gel with low concentrations up to 0.05~0.25%	Gellan gum offers proficient gelling
Quite stable at high temperatures and has a low pH value	Gel strength is not greatly affected by heating or sterilization, acid gel has relatively longer shelf life
Gel produced by Na^+ as well as K^+ can be replenished by heating, whereas that produced by Mg^{2+} and Ca^{2+} cannot be renewed	Can formulate both reversible and irreversible gel
Possesses outstanding ability to discharge flavour	Enhances the quality of the product
Can be utilized in combination with other gums such as starch, mixture of xanthan gum/locust bean gum	Gums produced with the help of gellan gum have structures that can be altered from brittle to elastic

2.4.2. Low Acyl Gellan Gum Structure

Gellan gum is composed of a tetrasaccharide unit which recurs and contains two β -D-glucose, one β -D-glucuronic acid and one α -L-rhamnose residue. These molecules are connected to form the structure exhibited in Figure 2.1. Gellan gum is originally found in the form of high acyl gellan (H) comprising O-5-acetyl and O-2-glyceryl groups on the (1-3)-linked glucose residue (as shown in Figure 2.1(a)). As shown in Figure 2.1, Gellan gum becomes deacylated, both acyl groups are hydrolyzed and low acyl gellan gum (L) is obtained when the gellan gum is treated with alkali at elevated temperature.

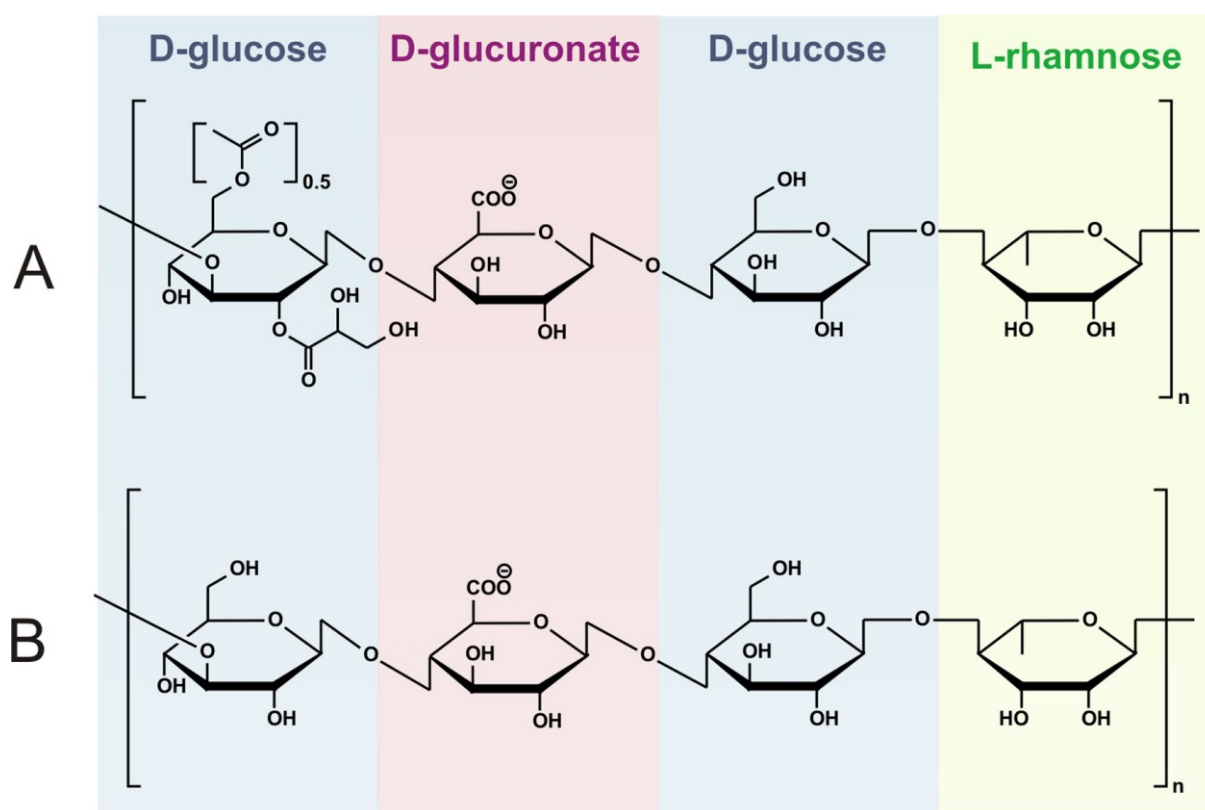


Figure 2.1 The chemical structure of gellan gum: (a) native form (high acyl); (b) deacylated form (low acyl). **Source:** Adopted from Saha and Bhattacharya (2010)

2.4.3. Low Acyl Gellan Gum

Hydrocolloids are a heterogeneous group of long chain polymers (polysaccharides and proteins) having a property of forming viscous dispersions and/or gels when dispersed in water e.g., Gellan gel (Saha & Bhattacharya, 2010). The main reason for the use of hydrocolloids in the food industry was the capability of modification of the rheology in the food system. The two main properties were flow behaviour (viscosity) and mechanical solid property (texture). The change in the texture is a useful ability to be used as food additives in the food industry. The functional properties of hydrocolloids in food include thickening, gelling, emulsifying, stabilization, control of crystal formation and sugar. The most extensively used properties are thickening and gelling (Saha & Bhattacharya, 2010).

Low acyl gellan gum is a biopolymer which fulfills the purposes of gel-forming, texturizing, stabilizing, film forming, suspending and structuring in food industry (Saha & Bhattacharya, 2010). Many countries use it as a food additive. It finds application in the processing of a large number of food products, such as confectionary, jams, jellies, dairy products and water based gels (Sanderson & Harris, 1990). The use of gellan in food products was permitted in 1992 in USA, followed by its acceptance in Europe as E418.

Gellan gum's excellent rheological properties make it a bacterial polysaccharide that has immense applicability in industries like food, pharmaceuticals (Osmałek *et al.*, 2014), and especially environmental bioremediation. According to the reports, it can be used in the bioremediation of polluted soils and aquifers (Moslemy *et al.*, 2004; Johnsen & Karlson, 2004). Low acyl gellan gum is also used enhance the property

of moisture retention, flavour release and storage stability in puddings and also to reduce syneresis (Sworn, 2000). The fluid like character of gellan gum at lower concentrations (Sworn *et al.*, 1995) can be used for the preparation of 'fluid-gels' that can be used in beverage industries. This gellan gum also acts as a very good stabiliser in reconstituted vegetable juice (Liang *et al.*, 2006).

2.4.4. Previous studies on Low Acyl Gellan Gum

This section critically reviews the previous studies that have been conducted extensively on Low Acyl Gellan Gum including industrial and research development over the last 20 years. 'Low acyl gellan gels form three folded double helical structure in an appropriate aqueous environment (Chandrasekaran *et al.*, 1988). Low acyl gellan gums are water soluble polysaccharide, which is used widely in food industry. The sectors such as dairy products, jams and jellies, confectionery and water-based gels were extensively using low acyl gellan gel gums (Sanderson, 1990). Low acyl gellan gum (highly deacetylated) (Sanderson & Clark, 1983). In the presence of cations these gels form brittle and hard gels which are based on the valence and concentration of cations (Grasdalen & Smidsrod, 1987; Moritaka *et al.*, 1991). In addition presence of co-solutes might also affect the properties of gellan gels (Evageliou *et al.*, 2010). Commercial low acyl (LA) gellan gum is produced by removing the acetate and glycerate groups with a strong alkali treatment.

Low acyl gellan gums produce non flexible, firm and brittle films (CP Kelco). The low acyl gellan gums are sensitive to ionic environment and divalent ions during the evaporation process. This is due to the properties of low acyl gellan gels which

include setting temperature, gel strength and melting temperature, which can be maintained by altering the cation and pH (Yamamoto & Cunha, 2007). The concentration of the polymers used, ionic environment, setting temperature and presence of other cations in the solution conforms the structure of the gellan gums (Yuguchi, *et al.*, 1993; Milas, *et al.*, 1990; Ogawa 1999). In the absence of the cation the low acyl gellan gum gels around 30C. According to Sanderson *et al.* (1988) the intermediate textural properties of high and low acyl gellan gels will obtain when combining low with high acyl gellan to form mixed gels.

Polysaccharide is recovered directly from the fermentation broth yield high acyl gellan gum; however deacylation by alkali treatment results in the low acyl variant (Gibson & Sanderson, 1997). The intermediate mechanical properties between those of the individual components are seen when combining low acyl gellan with high acyl gellan to form mixed gels (Sanderson *et al.*, 1988). It is widely reported that the low acyl gellan is acid sensitive, whereas it is not in the case of high acyl gellan form (Norton *et al.*, 2011; Yamamoto & Cunha, 2007).

In recent years, the biopolymer low acyl gellan gum has gain an attraction of tissue engineering. In tissue engineering it is used as a cellular scaffold due to its resemblance with the natural extracellular matrix (ECM) along with its bio-inert (Smith *et al.*, 2007; Oliveira *et al.*, 2010, 2011). The gellan gum has also been used as an injectable and printable matrix for cellular therapies and 3D tissue scaffold fabrication (Oliveira *et al.*, 2010; Pidcock, 2012; Ferris *et al.*, 2013). There is therefore potential for gellan gum based materials to be used for computer aided tissue engineering (Sun *et al.*, 2004; Ferris *et al.* 2013). The following section focused on.

2.4.5. Application of Low Acyl Gellan Gum in Food Industry

In food industry, Gellan gum is widely recognized as a food additive which effectively stabilizes, thickens, structures and forms gel in a range of food products. It also has a rapid setting behaviour, sparkling clarity of the gel and good flavour release and hence used as a preferred gelling agent for food products (Valli & Miskiel, 2001). It imparts diverse textures in food products, from stiff and brittle to soft and liquid-like. Some processed foods that are usually added with gellan gum are: dairy products, microwavable foods, jams and jellies, low-fat spreads, bakery fillings, confections, icings and glazes, sauces, dessert gels, frostings, structured foods, toppings and puddings.

Water-based dessert gels are globally celebrated because of their textural diversity. The ingredient responsible for the variety of textures and exceptional flavour burst is the gellan gum (Carrageenan, 2000; Saha & Bhattacharya, 2010). Adding minute quantities of gellan gum in gelatin desserts can enhance their heat stability and increase their setting temperature so as to allow the gels to set without being refrigerated (Karim & Bhat, 2008). Moreover, the gelled products that readily melt even at ambient temperatures are added with gellan gum to make them stable at these temperatures. Gellan gel can also be combined with xanthan or locust bean gum to produce ready-to-eat (RTE) dessert gels.

Special meat, fish and vegetable products are added with savoury gels or aspics to make them extra appetizing and succulent. The properties of the aspic can be enhanced by adding gellan gum completely or partially in place of gelatine. The addition of low acyl gellan in the gelatine gels has shown to increase the gelation

rate constant. Gellan gel forms coupled networks with the gelatine molecule and the anionic domains of the gellan forms new heterolytic junction zones with cationic domain of the gelatine molecules and this leads to the increase in gelation temperature and rate and gel strength (Fonkwe *et al.*, 2003). In addition, Lee *et al.*, (2004) mixed pigskin gelatine with gellan to obtain a composite film for packing or coating food materials.

Gellan gum is added to the products made up of fruits to impart strength during processing. Also, it enables the product to show greater firmness during conveyance and storage. Moreover, the product gets a tempting look along with a rich taste because it gives the distinctive gel texture even when added in small proportions (Tapia *et al.*, 2008; Saha & Bhattacharya, 2010; Falguera *et al.*, 2011). Gellan gum is also used in fruit fillings for bakery items, to give them shape and minimize starch levels. Fillings preserve water and demonstrate superior bake fastness due to the improved shape and the ability of the pastes to restore the structure to some extent after shearing and coating (Kohajdová & Karovičová, 2008). Gellan gum can partially replace starch and increase the flavour in bakery fillings (Saha & Bhattacharya, 2010).

Gellan gum is frequently used in the production of high solids products such as gelled confections without process alterations, because the formation of such products involves heating and cooling (Saha & Bhattacharya, 2010). Furthermore, gellan gum can be used along with suitable starch to lower the required setting time of starch jellies (Karim & Bhat, 2008). This enables them to get out of the starch moulds faster. Gelatine confections when supplemented with gellan gum can exhibit

greater heat stability, allowing the storage of these products at high atmospheric temperatures (Saha & Bhattacharya, 2010).

Gellan gum gives excellent shelf stability, moisture preservation, spread ability, lustre, texture and richness of flavour to the exquisite icings, frostings and glazes for bakery items (Kohajdová & Karovičová, 2009). In South East Asia, the texture of food is as significant as the taste of food. One of the many famous products produced by using gellan gum fluid technology is the drinking jellies. Moreover, it would be wrong to think that gellan gum is only a gelling agent. It is readily used in emulsions, cakes and other products that need shape and solidity, and not gelling (Kohajdová & Karovičová, 2009). The canned cat and dog food may also contain gellan gum. The textural diversity offered by a gelling additive is one of its most attractive features. Low acyl gellan gum can cause the gels to be hard and brittle, whereas high acyl gellan gum can impart softness and elasticity in gels (Lau *et al.*, 2000).

2.4.6. Mechanical Properties of Gellan Gum

Gellan gum offer different mechanical properties in each of its two forms i.e. acetylated and deacetylated. The fully deacetylated form (low acyl) is the hard and brittle phase whereas acetylated (high acyl) is soft and elastic. Also, with varying degree of deacetylation of polysaccharide, a number of diverse gels can be formulated. When low acyl gellan is dissolved in water at temperature above 90 °C and cooled to the setting temperature, the solution turns into gel owing to the existence of cations. High acyl gellan solutions set at a higher temperature than low

acyl gellan solutions. Since a distance is maintained between the polymer chains of gellan in double helix form and the bulky acetyl and glyceryl groups, the strength of high acyl gellan gels is quite low.

2.4.7. Gellan Gum Gel Formation

Gellan gum is polysaccharide secreted by the microbe *Sphingomonas elodea* (formerly *Pseudomonas elodea*) (Kang *et al.*, 1982). This polymer consists of d-glucose, -d-glucuronic acid and -l-rhamnose in the molar ratios of 2:1:1 (Sanderson, 1990). These monosaccharides are linked together in a linear primary structure. The native polysaccharide is high acyl gellan that contains O-5-acetyl and glyceryl groups on the 1 3-linked glucose residues. The acyl groups get hydrolysed and form a deacylated structure namely the low acyl gellan gum when exposed to alkali at high temperatures (Mao *et al.*, 2000).

The formation of the gel is a two step process. Gellan coils randomly to form a double helical structure and consequently aggregate to form a three-dimensional network in an aqueous environment and form junction zones (Huang *et al.*, 2004). The cations both mono and divalent present stabilizes the network by cross-linking the double helical structure through the carboxylate group. The divalent cations (M^{++}) cross-link the double helices directly, while monovalent cations (M^{+}) cross-link double helices indirectly through the medium of water (Chandrasekaran *et al.*, 1992; Chandrasekaran & Thailambal, 1990). The formation of gellan gels happens in the presence of either mono or divalent cations by cooling the hot polymer solutions (Sanderson, 1990) through weak interactions namely hydrogen bonding and van der Waals forces (Matsukawa & Watanabe, 2007; Nickerson *et al.*, 2003; Picone &

Cunha, 2010). The gum formation in the case of the low acyl gellan gum is also initiated by pH reduction or through the cations. The cations bind at the site of the carboxylate groups on the neighbouring helices or by restraining the electrostatic repulsion by binding to single helices (Morris *et al.*, 2012). These gels are non-elastic, hard, brittle and transparent gels (Ogawa *et al.*, 2005). The formation of these gels requires a high polymer concentration and a long setting time at low temperature (30-50°C). Adding the divalent cations such as Ca^{2+} or Mg^{2+} promotes the gelation (gel formation) of aqueous solutions and the rheological behavior becomes less temperature dependent (Moritaka *et al.*, 1991; Moritaka *et al.*, 1992).

The gel formation and melting temperature shifts from low to high temperature and the number of junction zones are increased in the presence of salt ions. Hence, making the gel more heat resistant and leading to an enhancement in the gel elastic modulus and rigidity (Horinaka *et al.*, 2004; Mao *et al.*, 1999; Moritaka *et al.*, 1995; Yamamoto & Cunha, 2007).

In both representations, filled circles symbolize cations that encourage the collection of gellan double helices.

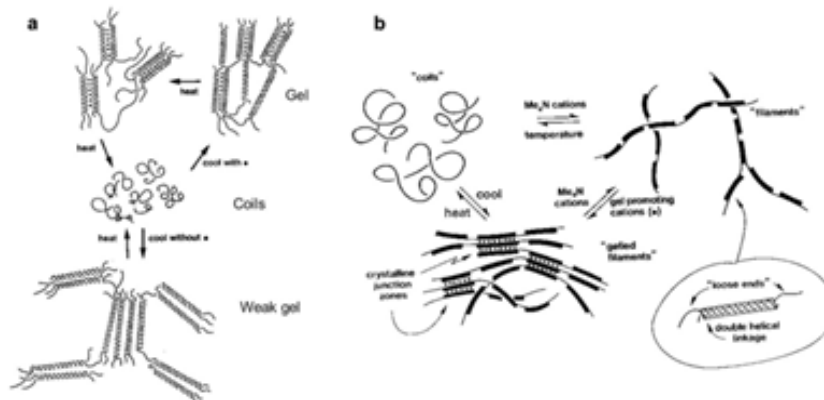


Figure 2.2 Models for gelatin of gellan put forward by (a) Robinson et al. (1991) and (b) Gunning and Morris (1990). In both representations, filled circles symbolize cations that encourage the collection of gellan double helices

2.5. Gel Texture

Texture is considered as one of the primary determinants of the food quality. Majority of the investigations were conducted to get a better 'know-how' regarding the texture of the food have been conducted at high deformation by means of the texture profile analysis (TPA) (Lau *et al.*, 2000). The majority of the texture concept is developed based on the food disintegration mechanism during mastication (Renard *et al.*, 2006) while the path of disintegration can be understood mainly in the light of mechanical properties and structural changes of food (van den Berg *et al.*, 2007). From various studies it is evident that almost all the food products also have certain mechanical properties (Barbosa-Cánovas *et al.*, 2004/Rev.2006). Communication is one of the widely used food processing techniques and is related to fraction. A person senses the food texture when he chews a certain food product before swallowing it. Food texture can be controlled by achieving an optimum combination of mechanical and fractural properties (Funami, 2011). It is hard to define and describe food texture completely since it is dependent on various factors. The term texture has diverse

implications, but in researches related to foods it is defined as the aggregate of mechanical, structural and acoustic properties which is recognized by a consumer as a physical trait of the food. A great number of foods are intricate and complex in terms of mechanical properties. Moreover, variations in moisture content, temperature and pH leads to changes in the properties of food when it is being chewed.

Following are some important definitions of the mechanical properties related to the food texture:

- Hardness can be defined as the maximum force during the first compression cycle;
- Brittleness is the ratio of the first considerable fracture during the first compression cycle and the original height of the sample. It is given as a percentage. A lower value of brittleness signifies a higher degree of brittleness in gel;
- Cohesiveness is expressed as the area of the second compression divided by the area of the first compression;
- Modulus can be described as the supposed firmness when the gel is compressed slightly. It is similar to squeezing a fruit gently to check its ripeness;
- Fracture strain is the degree of compression that can be tolerated by the gel before it ruptures. The material is said to be highly brittle if it has a low value of fracture strain;
- Springiness can be defined as the detachment the sample was compressed during the second compression to the peak force, divided by the initial height of the sample. It is presented in the form of a percentage;

Sanderson *et al.* (1988) initially studied the texture of high and low acyl gellan gels. The author reported that high-acyl gels are non-brittle and elastic that is similar to the mixed gels of xanthan gum and locust bean gum. Baird *et al.* (1992) and Morris *et al.* (1996) also studied the texture of gellan gum and found that the progressive removal of acyl groups from the high acyl gellan chain, the mechanical and gelling properties became similar to the low acyl gellan gels. Mao *et al.*, (2000) suggested that the weight ratio of high acyl to low acyl gellan plays a major role in controlling the gel texture properties rather than the total polymer concentration. Similar texture studies using texture analyzer and rheometry were studied by Huang *et al.* (2004), Mao *et al.* (2000, 2001) and Tang *et al.* (1995, 1996).

2.6. Gel Strength

The strength of the gellan gel produced is dependent upon a number of factors, such as gellan concentration, valency and concentration of cations employed in gel formulation (Jampen *et al.*, 2001). A greater quantity of monovalent cations needs to be added in order to obtain the gel strength similar to that obtained when divalent cations are used. The gel is the strongest when a greater number of cations are present in it; this is the case for all types of mono and divalent cations (Tang *et al.*, 1997). The stoichiometric relations between the number of cations and the number of carboxyl group present help determine the maximum gel strength (Sanderson & Clark, 1984; Tang *et al.*, 1997). Furthermore, Tang *et al.* (1997) stated that when the stoichiometric uniformity exceeds, a contest for anionic places starts, inflicting repulsive forces causing the gels to have poor strength.

2.7. Water Holding Capacity (WHC)

A great quantity of water is retained by gel structures. Gels are characterized on the basis of their water retention ability. A common definition of WHC is the amount of water which can be held by the gel structure, or ability of a gel to preserve this water when stored or subjected to externally applied loads. The gelation mechanism helps to identify the WHC property a gel. WHC is a significant determinant of quality when it comes to food gels because it has an impact on the textural properties and cost of the food product (Huang *et al.*, 2003).

Also, Lau *et al.* (2000) declared that variations in WHC ability of the low acyl gellan gels can result in the simultaneous formation of two different types of microstructure

within gellan gels. The first microstructure makes the gel stable for long periods of time. This also implies that the water holding capacity and texture stability after prolonged storage of gels having this microstructure are irrespective of cation concentration. The second microstructure plays a part in how the gel behaves when subjected to external loads. Such a microstructure depends upon the cation concentration. SEM micrographs were used to verify the presence of these two types of microstructures. Also, the gels were found to have two different pore size distributions. The thick strands of gel structure formed large pores whereas thin web structure caused a smaller pore size to exist. The much smaller pore size enables the thin web structure of gels to confine the water in them due to the greater capillary force (Mao *et al.*, 2001). The quantity of water may be lost during long storage time due to passive diffusion (Sanderson, 1990). Water can also be lost due to external forces such as freeze thawing, temperature fluctuation. This results in the shrinkage, reduction in quality and change in the texture (Mao *et al.*, 2001). Hence, WHC is an important factor for evaluating the acceptability of the food gel.

2.8. Introduction to Mechanical Properties

2.8.1. Stress Definition

Stress can be defined as the external expression of the restoring force, which is generated when the inter-atomic bonds that hold the material intact are stretched. In some cases, the term deformation is replaced by 'displacement' and the term 'load' is used as a substitute of 'force'. Stress is expressed as force per unit area and is

denoted by(σ) its unit is same as that of pressure that is Pascal. Following is the formula for stress:

$$\sigma = \frac{F}{A} \quad (2.1)$$

where,(σ)is the symbol for stress experienced when a force (F) is applied uniformly over a body having a cross-sectional area of (A).

Generally, the force is divided by the sample's original area(A_0); however in case of significantly large strains, one can apply the instantaneous area (A) at any point. Depending on the loading conditions, diverse stress states are described. For instance, if the load applied is intended to decrease a dimension of the sample, it is called compressive loading. Similarly, if the force is intended to elongate or compress the material in one direction; it is termed as uniaxial tensile or compressive force. (Dobraszczyk & Vincent n.d.)

2.8.2. Strain Definition

Strain can be defined as the change in dimension or shape of a material when it is under the effect of stress. Since strain is a ratio that compares the shape prior to and after deformation, it is pure and has no dimension. It is given as the change in dimension over the initial dimension, such as increase in length (ΔL) or extension divided by the original length,($ln \frac{L}{L_0}$) called the Hencky strain (ϵ_H). The two values are quite similar up to a strain of 10%, but a strain greater than that causes considerable deviation. Practically, engineering strain (e) is used for majority

of load-bearing materials, which mostly undergo small strains during operation. However, engineering strain fails to correctly define strains in extensible materials. The Cauchy strain equation can be effectively applied to small elastic strains in case of small overall elongation, but in case of materials that undergo large strains, the original length varies significantly, and the usual engineering equation is insufficient since it depends completely on the original dimensions. Majority of the food materials experience considerably large strains in excess of 100% and Hencky strain (ϵ_H) describes them more appropriately, as it considers the incremental change in dimension (ΔL), called the instantaneous length during stretching (L) (Dobraszczyk & Vincent n.d.) (L_0), called the engineering or Cauchy strain (e) or as the natural logarithm of the

$$\text{Cauchy or engineering strain} \quad e = \Delta L / L_0 \quad (2.2)$$

$$\text{(Hencky strain)} \quad \epsilon_H = \int_{L_0}^L \frac{dL}{L} = \ln \frac{L}{L_0} \quad (2.3)$$

Following is the equation to represent the relationship between Hencky and engineering strain:

$$\epsilon_H = \ln \frac{L}{L_0} = \ln(1 + e) \quad (2.4)$$

2.8.3. Young's Modulus Definition

Young's modulus of elasticity can be defined as the stress to strain ratio, when an elastic solid material is subjected to tensile or compressive forces. It is also called 'Modulus of Elasticity'. It is the function of stiffness and is expressed as:

$$E = \frac{\text{stress}}{\text{strain}} = \frac{\frac{F}{A}}{\frac{\Delta L}{L}} \quad (2.5)$$

where (E) denotes Young's modulus, (F) represents the force applied perpendicular to the area considered by the stress, (A) symbolizes the cross-sectional area of the specimen, (L) implies the length or height of the specimen and (ΔL) is the change in length caused under the effect of force (F). The slope of the stress-strain curve is proportional to Young's modulus.

2.8.4. Strength Definition

Strength refers to the stress required to break a material. Although strength is similar to stiffness in ways like it is affected by time, temperature, and other parameters and both have the same direction, but strength and stiffness are not the same phenomenon. Strength can be gauged in a variety of ways but standard tensile or compression test is the simplest and most effective of all, giving the value of tensile strength and compressive strength. The unit of strength is Pascal (Pa) (Lu & Abbott 2004)(Dobraszczyk & Vincent n.d.). Also, there is some confusion in strength and toughness as well and they must be distinguished. Strength is basically the maximum stress endured by an object before fracture, whereas toughness is the resistance to cracking. In the light of the force-extension relationship, strength is expressed simply as the ratio of maximum force and the cross-sectional area. On the

other hand, toughness refers to the energy needed for fracture propagation on a specific crack site. It is usually measured as the area under the force-extension curve. This implies that it is not necessary that a strong material will always be tough. Consider the example of ceramic and glass, both these materials are strong (a greater force required to break them) but they have toughness lower than wood, which fractures at a lower force but deforms greatly before it. Human tendon and muscles are even weaker, but they are relatively tougher because of their large extensibility (Dobraszczyk & Vincent n.d.).

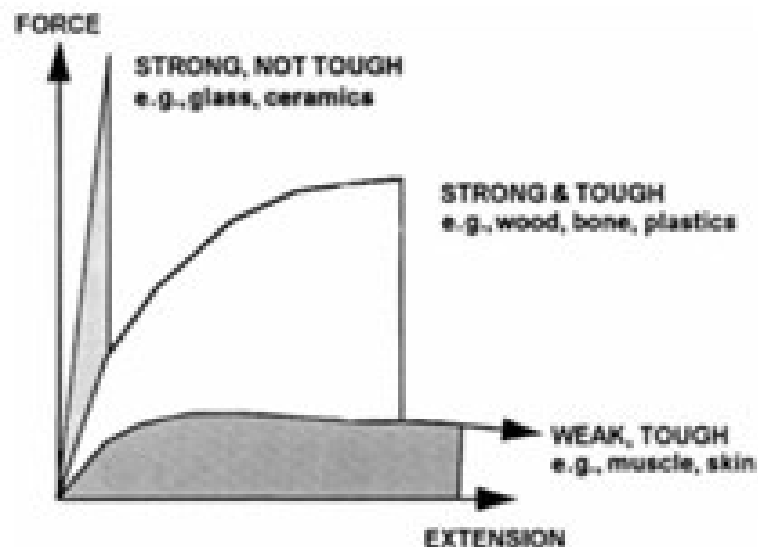


Figure 2.3 Normal Force-Extension plot illustrating different materials having contrasting strength and toughness (Dobraszczyk & Vincent, n.d).

2.8.5. Work and Energy at Failure Point Measurement

Not many instruments are designed on the principle of work and energy. The dimensions for both energy and work are ($mass \times length^2 \times time^{-2}$) which is equal to ($force \times distance$). Instruments used to plot out the force-distance curve

as a test result can offer work functions either by computing the area under the force-distance curve or by any other method that provides the force-distance integral. One must keep in mind that a compression-decompression cycle contains two work elements: (1) the area under the curve during compression loading signifies the work done by the machine on the gel and (2) the area under the curve during unloading signifies the work restored by the machine during the recovery of the gel. Usually, the work done during unloading is small but to achieve precise results it is important to distinguish the two factors.

A force-distance curve can be obtained by the Instron texture analyzer, the Food technology Texture Test System, and similar universal testing machines. The area under this curve can be transformed into real amount of work.

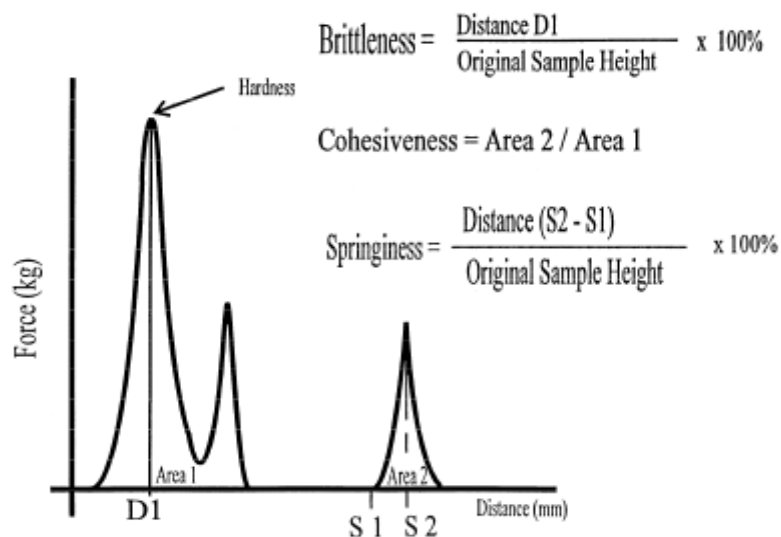


Figure 2.4 Typical instrumental texture profile analysis curve for gellan/gelatine mixed gels (Lau et al. 2000)

2.8.6. Relationship between Fracture Mechanism and Energy

While studying the fracture mechanics, it is typically supposed that all materials are inhomogeneous; they contain fine (almost invisible) cracks or in-homogeneities (defects) which in effect act as minute cracks. Propagation of any of these fine cracks may eventually lead to fracture. Crack propagation occurs when the following two requirements are fulfilled:

- 1) The stress at the tip of a crack must be greater than the cohesion or adhesion stresses observed between the structural components. This will be the crack initiation condition (beginning of the fracture).
- 2) The differential amount of strain energy dissipated as the crack propagates is greater than the differential amount of energy required. This will lead to spontaneous crack propagation (fracture propagation).

A specific amount of energy is required to deform a material. This externally supplied deformation energy can be stored (elastically), utilized for fracture propagation, or given out in some other way. Dissipation of energy can occur due to two processes. In first process the flow of the entire material is viscous, i.e. it behaves in a visco-elastic manner like cheese, meat and bread dough. In second process, energy is given out as a result of the friction between two or more constituents of the material. The friction occurs due to inhomogeneous distortion of the material at large strains. The distortion and friction between fillers and continuous phase in the second process causes the liquid to flow through a gel matrix. Theoretically, any material

which is heterogeneous on a larger degree than molecular will give off energy due to friction.

The general expression for the energy balance during deformation and fracture is given as:

$$W = W_e + W_{d,v} + W_{d,c} + (W_f) \quad (2.6)$$

where (W) represents the energy supplied to the material; (W_e) denotes the portion of the strain energy which is elastically stored; ($W_{d,v}$) is the energy consumed because of visco-elasticity; ($W_{d,c}$) is the energy spent as a result of friction and (W_f) is the energy used to fracture. The initial value of (W_f) is zero and is derived (chiefly) from (W_e). Fracture in a material proliferates when the value of (W_e) is so high that the energy dissipated because of stress relaxation in the material is near the budding crack and surpasses the value of (W_f). Brittle behavior probably suggests that the values of ($W_{d,v}$) and ($W_{d,c}$) are (extremely) small and thus allow greater crack propagation along with the generation of shock waves at speed of sound during procreation of fracture (van Vliet 2002).

2.9. Effect of Additive Materials on Low Acyl Gellan Gum Structure and Gelation

2.9.1. Effect of Salt (cations) Concentration on Low Acyl Gellan Gum Structure and Gelation

Past researches on gellan (Sanderson & Clark 1984) and a lot of studies discussed below (Kasapis et al. 1999) (Moritaka et al. 1991) (Sanderson & Harris 1990) have demonstrated that the strength of gel increases at first when salt is gradually added to it but when the salt concentrations surpasses a critical level the strength decreases. Figure 2.5 illustrated this phenomenon for mixture of 0.8 wt% K^+ gellan with KCl and of 0.3 wt% Na^+ gellan with $MgCl_2$ (Chandrasekaran et al. 1988). In both cases, the maxima is shown in Young's modulus (E) and force at break (proportional to breaking stress(σ_b)); however, the molar concentration at which these take place are 30 times greater for K^+ in comparison to Mg^{+2} . In case of both the salts, the maximum in break stress is observed at notably lower salt concentration in comparison to the maximum in modulus. Similar findings were reported for Na^+ gellan added with NaCl while comparing the salt-dependence of (σ_b) with storage modulus (G) (Morris et al. 1999).

The research conducted by (Milas & Rinaudo 1996) suggested that an increase in the concentration of added KCl or $MgCl_2$ results in three different types of fracture mechanisms exist when the material is subjected to compressive forces. Figure 2.5 is the schematic representation of this finding, in which the zones of salt concentration over the fracture took place are specified as a, b and c. At Zone a, which represents the salt concentration below the maximum in break stress, the gels showed poor strength and experienced a brittle fracture along a single fracture plane. The gel formed at zone b is stiffer and this zone represents the area around salt concentrations between the maximum in break stress and the maximum in modulus. In this case multiple fine cracks were observed during fracture. At zone c, where the salt concentration is above the maximum in modulus, the softer gels were

formed and when they fall apart it appeared like a coagulation or phase-separation process.

Biopolymer gels represent a portion of various solutions of single molecules at one extreme to highly-compacted solid to the other as the degree of inter-molecular interaction is amplified (by changing in variables like pH and ionic environment). Ideal cross-linking will take place at some point within the range; less interaction will lead to a weaker structure whereas more interaction will produce larger agglomerates. This will be followed by a decrease in the effective number of single junctions, until at last the structure disintegrates to form a solid precipitate. The description is in accordance with the apparent phase separation reported for gellan gels at extremely low pH values or with a high salt concentration (Zone c in Figure 2.5), and with the finding (Ohtsuka & Watanabe 1996) that the gels starts turning viscous when their strength begins to reduce.

The effect of NaCl, KCl, MgCl₂ and CaCl₂ concentration on the gelation of the gellan gel has been studied extensively by Izumi *et al.* (1996), Kang and Pettitt (1993), Miyoshi *et al.* (1996) Morris (1990) Tang *et al.* (1995, 1996, 1997) and Totosaus *et al.* (2009). These levels of cation concentrations play a major role in the modification of the gels. For example, lower levels of CaCl₂ provide a stronger gel promoter than MgCl₂. The higher levels of calcium ion in the gel produce a strong repulsive forces leading to the decrease in the strength of the gel (Tang *et al.*, 1995). Izumi *et al.* (1999) reported that the calcium ion has a better effect on the enhancement of gellan gum gelling ability than the magnesium ions. Biopolymer gels represent a portion of various solutions of single molecules at one extreme to highly-compacted solid to the other as the degree of inter-molecular interaction is amplified (by changing in

variables like pH and ionic environment). Ideal cross-linking will take place at some point within the range; less interaction will lead to a weaker structure whereas more interaction will produce larger agglomerates. This will be followed by a decrease in the effective number of single junctions, until at last the structure disintegrates to form a solid precipitate. The description is in accordance with the apparent phase separation reported for gellan gels at extremely low pH values or with a high salt concentration (Zone c in Figure 2.5), and with the finding (Ohtsuka & Watanabe, 1996) that the gels starts turning viscous when their strength begins to reduce.

It can be conclude that by saying that the transformation of the polymer from the haphazard coil state into the double-helix form is the most initial step of gelation of gellan. Still, conformational ordering fails to provide a consistent set-up. To form original gels, conversion of double helices into steady agglomerates is required. Electrostatic repulsion between the helices hinders agglomeration. Repulsion reduction through promoting gel formation can be performed by decreasing the pH, which in turn brings down the charge on the helices as the glucuronate carboxyl group changes from the negatively-charged COO^- form into the uncharged COOH form (Lau *et al.*, 2000). Salt can also play a role in encouraging agglomeration. The plain anions and cations present in the added salt shield electrostatic repulsion between the gellan helices. Cations form aggregates around the helix, which decreases the repulsion even more, hence causing a reduction in their effective negative charge (Milas & Rinaudo, 1996). However, both of these approaches are imprecise: charge screening is completely dependent upon ionic strength, measured via the concentration and charge of both anions and cations, and the degree to which the (positively charged) cations preferentially surrounds the (negatively

charged) helices is estimated by the charge and the concentration of the cations. On the other hand, alkali metals cations can lead to further decrement in the effective negative charge of the gellan double helices by adhering to them at particular interactive regions. At first site binding is activated with the help of electrostatic attraction between cations and the carboxylate groups of the polymer, but it starts to amplify and become constant with the development of a harmonization network with suitably-spaced oxygen atoms from both strings of the double helices (Mao *et al.*, 2000; Nishinari & Takahashi, 2003).

Gellan double helices becomes steady as they link together to form clusters. This results in thermal hysteresis between the temperatures which cause the helices to form upon cooling and the clusters to melt on heating. In case of gelation containing alkali metals cations, the start of hysteresis is followed by the beginning of production of original gels, and takes place as the cation concentration is lowered gradually and ionic size becomes bigger. The mechanical spectra exhibited by solutions of ordered gellan at low concentration of monovalent cations is comparable to those of solutions of randomly arranged coils. As the salt concentration is augmented any further a zone with weak gel response is formed before the threshold concentration for production of real gels is attained. This development is noted in case of alkali metals and is a result of the continuous inhibition of electrostatic repulsion within the gellan double helices (Morris *et al.*, 2012).

According to (Chandrasekaran *et al.* 1988) the hardness of the gel depends upon a critical value of cation concentration. When the cation concentration is below that level an increase in hardness is noted whereas the hardness decreases if the cation concentration is above that level (Lau *et al.* 2000). The findings of (Sanderson &

Harris 1990) and (Tang et al. 1994) showed that gel strength in gellan gels decreased when cation concentrations were above the critical value. Tang et al. (1994) proposed that a larger quantity of cation ions might take the places of anions in gellan gum molecules, thus restricting the cross-linking of adjoined polymer chains. Consequently, repulsive force in the junction zones may minimize the cross-linking between the collections of helices, forming a gel with a weak structure. As stated by (Kasapis et al. 1999), a large number of ordered number of nuclei are produced in the hot sol due to the excess cations, which generates a low functionality system upon gelatin (Lau et al. 2000). Moreover, Lau et al. (2000) suggested that the cations concentrations play a vital role in making a gel brittle; the higher the concentration of cation, the more brittle the gel will be.

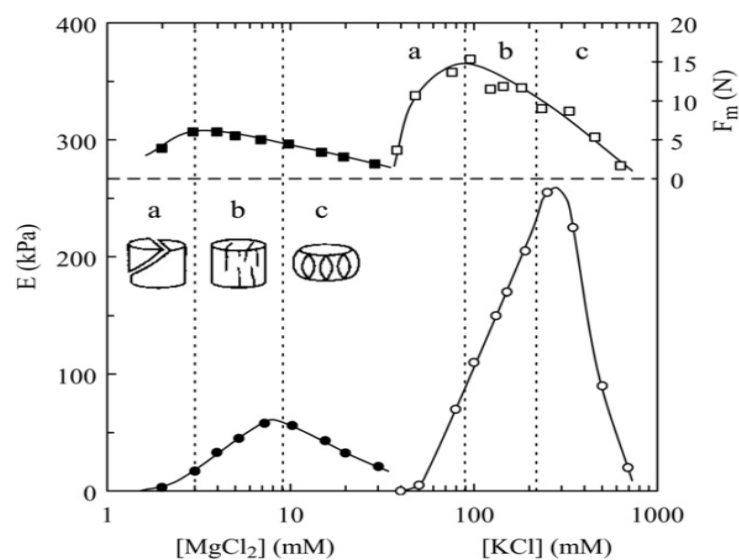


Figure 2.5 Variation in Young's modulus, E (circles) and maximum force (F_m) at fracture (squares), as a result of compression testing (25 mm/min) of cylindrical samples (17 mm height, 17 mm diameter) at 25°C, upon inclusion of MgCl_2 to 0.3 wt% Na^+ gellan (filled symbols) or KCl to 0.8 wt% K^+ gellan (unfilled symbols). The diverse failure mechanisms examined in regions a, b, and c for each salt, are illustrated schematically between the traces for MgCl_2 (from Milas & Rinaudo, 1996).

2.9.2. Effect of Gellan Concentration on Low Acyl Gellan Gum Structure and Gelation

The conclusions drawn by (Yamamoto & Cunha 2007) in their research are that the gellan chains are in close proximity to each other; this increases the chances of agglomeration and development of junction zones. Also, lesser time is taken by the gel to set and a rather compact network structure is achieved having bulkier strands that lead to harder and viscous gels. Furthermore, the compaction of the linked network enabled the applied stress to be distributed uniformly and thus gels with higher concentration have higher failure strain or deformability. The networks get their strength from the minute pores and a large number of junction zones present in the matrix. Such structures are less prone to disintegration, even when loaded externally, which makes them have greater Water Holding Capacity (WHC) values (Yamamoto & Cunha 2007). The higher the gellan concentration, the higher the failure stress and energy required for compression. Thus, it can be concluded that more gellan holds more water to give a solid-like gel appearance, which is undoubtedly more resistant than gellan gels with low gellan (Banerjee & Bhattacharya 2011).

As illustrated in (Banerjee & Bhattacharya 2011) study, the maximum stress is an index which indicates the greatest level of resistance provided per unit contact area toward compression, whereas compression energy represents the amount of work/energy required to attain the investigational dimensional change. As we add an increased quantity of gellan gum, the maximum stress and compression energy increases as well. Since a greater degree of hydrocolloid can hold a greater amount

of water to form a solid-like gel, it apparently resists more than a low-level hydrocolloid gel (Banerjee & Bhattacharya 2011).

2.9.3. Effect of Sugar Concentration on Low Acyl Gellan Gum Structure and Gelation

To provide the required functional properties, food gels use sugars. For instance, high methoxyl pectin gels were created with a high concentration of sugars (da Silva & Goncalves 1994) (Ptitchkina et al. 1994). The Young's modulus and the melting point of k-carrageenan gels was increased after adding sugar (Tang et al. 2001) (Nishinari et al. 1990). The strength of k-carrageenan locust bean gum mixed gels was decreased by the sucrose, but the strength of alginate gels increased (Tang et al. 2001) (Fizman & Duran 1992). The increase in concentration of sugars led to the increase in the dynamic elastic modulus of agarose gels, which however decreased when sugar was added in excessive amounts. Enough material is available for review upon the ability of high concentration of sucrose or other sugars to induce gelation of high methoxy pectin under acidic conditions for the production of jams and jellies. The strength and thermal stability of networks formed by gelling biopolymers such as kappa carrageenan (Nishinari et al. 1990), starch (Nishinari & Watase 1992), oxidized starch (Evageliou et al. 2000), agarose and gelatine (Nishinari & Watase 1992) was also increased with the addition of sugar.

The effect of sugar on gellan used in concentrations required to form gels without using sugar have also been studied in other various papers. The effect of the concentration of sucrose on the mechanical properties of the gellan gels was studied through compression testing by (Bayarri et al. 2002) As the sucrose concentration was increased, a systematic increase was observed in Young's modulus (E), break

stress (σ_b), and strain at break (ϵ_b). Smaller changes were observed when using lower concentrations of gellan, but these effects were observable at higher concentrations. Similar effects as the cation were observed by Tang, Mao, Tung and Swanson for the stabilization of the orderly packing structure of gellan. The formation and development of junction zones was also obstructed with the excessive sucrose. Gellan gels were hence strengthened at low cations, but at high cation concentrations, the gels got weak due to sucrose (Tang et al. 2001).

2.10. Texture of Gellan Gels

General Foods initially developed the technique of texture profile analysis (TPA) in 1960s, which was later applied by Kelco at a larger scale to characterize gellan gels and to compare their textural properties with those obtained by using the gellan agents obtained through gelling agents (Gibson & Sanderson 1997) (Sanderson & Harris 1990) (Sanderson et al. 1988)(Ortega & Sanderson 1994) (Sworn et al. 2009). A free standing gel was exposed to consecutive cycles of compression in this process. The sample was compressed to 30% of its initial height at 2 inches per minute in the procedure conducted by Kelco (Sanderson & Harris 1990) A second cycle of compression was created under similar conditions to raise the crosshead of instrument.

The first compression modulus led to three parameters such as, modulus, hardness and brittleness. Although expressed in different units, the modulus is similar to Young's modulus (E). The maximum force created in resistance to the first compression is usually termed as hardness and is equal to the break stress (σ_b). After initial failure, maximum resistance may occur during compression in some

samples. It is at the break that the brittleness value is recognized to strain, which implies that very low brittleness values will be expressed by the brittle materials, while high brittleness will be shown by the very elastic materials.

The second cycle of compression will be used to get elasticity and cohesiveness as two more parameters. Elasticity is derived from the point at which the descending crosshead touches the top of the sample. The ability of a sample to recover from compression is termed as elasticity and is defined as the height of the sample at this point. It is expressed as a percentage of the initial height before compression. Toughness during eating is referred to as cohesiveness. The area under the force-distance curve obtained in the second cycle of compression is equal to the cohesiveness of a material. It is denoted as a percentage of the corresponding area from the first compression.

Gels strongly characterized by the compression testing with 0.2 wt% concentration of polymer used were found present in the commercial low acyl gellan gum. The progressive reduction in pH or progressive addition of cations led to an initial increase in the values of hardness and then a decrease, which was verified further by the later studies done under improved ionic conditions. The cation concentration led to a systematic decrease in the brittleness values.

Although agar itself is an effective gelling agent, (Sanderson et al. 1988) proved that gellan could be used at much lower concentrations to match the modulus, hardness, brittleness, elasticity and cohesiveness of agar in traditional Japanese food products. A progressive reduction in the brittleness values and a progressive increase in the

elasticity, modulus and hardness were observed after progressively increasing the concentration of gellan at fixed concentrations of the added cation.

2.11. Release

2.11.1. Flavour Release

Hydrocolloids are increasingly being used in the field of reduced fats and salt products, as health consciousness amongst the consumers is increasing (Prance, 2007). Consumers are demanding more information about the mechanical and physical properties of hydrocolloids as new products are being introduced with hydrocolloids in them. These also include the flavour increasing properties as well. Flavour will only be detected in the presence of highly concentrated compounds, while the flavour perceived can be changed by slightly varying the composition of the gel system. The mechanisms involved in the breakdown of the food matrix and specially the flavour releasing compounds, will benefit the food industry immensely (Bowen *et al.*, 2003).

Three dimensional hydrophilic, polymeric networks with chemical or physical cross links are termed as hydrogel, which have the potential to contain large amounts of water or biological fluids. Polysaccharides are extremely useful macromolecules as compared to the synthetic polymer, as it is found in living organisms and is also produced by the recombinant DNA. Polysaccharides have economical benefits as well. They are non toxic and biocompatible and display a number of physical and chemical properties that deems them suitable for various applications. The literature has studied the effects of rheological properties of hydrocolloids on the aroma and taste released during consumption. The increase in the viscosity will generally lead

to a decrease in the aroma and taste perception, but binding, mouth coating and fresh surface generation also play a part in its behaviour (Malone et al. 2003)(Darling et al. 1986). Guar gum concentration specifically above the critical concentration of the guar gum will affect the sweetness and strawberry flavour, states Baines and Morris. It was observed that the viscosity had to be increased by at least two orders of magnitude in order to obtain a threefold reduction in the flavour perception using the guar gum as a thickener. Flavour release from gelatine was observed by (Bakker et al. 1996) through the time intensity analysis. Gels with higher gelatine concentrations showed a slower rate of release thus implying that the rate at which the gels were broken down through melting and chewing would govern the rate of release. Alginate gels were used to observe the effect of cation concentration on flavour perception. The study was conducted by (Baines & Morris 1987). Until reaching the minimum flavour plateau intensity, it was observed that the increase in the cation concentration led to an increase in the gel strength. Aroma and taste could only be controlled through large rheological changes. Flavour perceptions were affected by the binding of taste and aromas to biopolymers and mouth coating behaviours of such thickeners.

As compared to soft or medium gels, the firm gels released flavours with lower maximum intensity states (Boland 2004). The mechanism of physical entrapment of flavour molecules within the food matrix and the mechanism of interactions between the flavour molecules and the gel components may be responsible for the effect of hydrocolloids on flavours. The transport of small molecules such as, flavour volatile molecules moving from the gel surface to the system, is obstructed by the presence of polymer network.

The breakdown of food was done through mastication, as demonstrated by (Harrison & Hills 1997). This resulted in the increase in the release of flavour volatiles as well as the surface area available for diffusion. Higher breaking strengths and lower perceived flavour intensities were experienced by the harder gelatine gels, states (Wilson & Brown 1997). The texture of the gels will affect the flavour release, which implies that the lowest flavour was released by the most rigid gels (Boland 2004). According to Boland (2004), the matrix flavour interactions led to differences in the flavour release and similarity in the texture of the gels.

2.11.2. Salt Release

Public health authorities are primarily worried about the salt content in food and are focusing on ways to reduce the salt in foods. Conditions of hypertension are a result of the dietary salt, which when consumed lead to cardiovascular diseases, gastric cancer, osteoporosis, cataract, kidney stones and diabetes (Elliott & Brown 2006). The daily salt consumption was reduced to 5 gr by the World Health Organization. Salt content higher than this is being allowed in many developed countries.

Organoleptic qualities may be compromised if the salt content is reduced, which means that the food manufacturers have yet another hurdle to cross before making low salt content mandatory in foods. Aroma and taste perception is reduced by the effect of food structure and texture (Phan et al. 2008) (Saint-Eve et al. 2009) (Koliandris et al. 2010) (de Loubens et al. 2011) The main target is to not damage the perceived saltiness of food, while also reducing the salt content.

More salt was released by the brittle gelatine gel during the two bite compression as compared to the soft elastic ones, states Koliandris *et al.* (2008). An inverse

relationship between the fracture strain and the salt release was also found by Koliandris *et al.* (2008). Increase in the viscosity or gel strength of foods also led to a decrease in the perceived taste intensity in the mouth, reports Ptitchkina *et al.* (1994). Acyl gellan gum, kappa carrageenan, calcium alginate, and the mixture xanthan. The release kinetics was not affected by the compression velocity, says (de Loubens *et al.* 2011), but breakdown took place when the compression velocity was increased from 1 mm/sec to 10 mm/sec (de Loubens *et al.* 2011). The study also revealed that by increasing the strain, the release of salt became fast, which further highlighted the role of strain level on salt release kinetics. The product breakdown will be more in the presence of a greater contact area when provided at higher levels of strain, which will also contribute this difference. The salt release would increase to a greater extent owing to the breakdown of product and an increasing contact area between water and product, although the strain level was higher than the failure strain salt release.

More salt was released by the brittle gelatine gel during the two bite compression as compared to the soft elastic ones, states (Koliandris *et al.* 2010). An inverse relationship between the fracture strain and the salt release was also found by (Koliandris *et al.* 2008). Increase in the viscosity or gel strength of foods also led to a decrease in the perceived taste intensity in the mouth, reports (Ptitchkina *et al.* 1994). Acyl gellan gum, kappa carrageenan, calcium alginate, and the mixture xanthan gum and locust bean gum was used in a test to see the effects of reducing the fracture strain of polysaccharide gels. The result was an increase in the perceived sweetness for the gel food types.

2.11.3. Correlation between Breakdown Properties and Release

There is scanty of literature related to micro structural properties of gellan gels on large deformation. Van den Berg *et al.* (2007) studied the physical properties such as serum release, energy storage during the deformation, fracture point after the breakdown of the gels. The energy dissipation was caused by the release of the serum during the breakdown of the gels.

The fracture point and the deformation of the gels by uniaxial compression is characterized by the true fracture strain), the true fracture stress (σ_t), and the energy to fracture (van den Berg *et al.*, 2008). The macroscopic breakdown of the gel is characterized by the curve following the fracture point. This helps in calculating the energy balance in the gels during the breakdown (Van den Berg *et al.*, 2008). Highly elastic gels store energy during the breakdown and hence have high recoverable energy values and also show a rapid breakdown. Coarse-stranded gels break into innumerable number of particles by showing many fractures.

Secondly, during the breakdown, the gels release different amounts of serum. Bi-continuous and coarse-stranded gels release the highest amount of serum. Instead, the smooth and homogenous gels release low amount of the serum and break down easily. The serum release also affects the size of the deformation and the fracture properties (Van den Berg *et al.*, 2007). This in turn changes the volume of the gels and the fracture properties. The release of the serum is directly related to the porosity of the gel. The increase in the deformation is indirectly proportional to the pore size and the connectivity. The permeable area of the gel also reduces with the

breakdown. The porosity of the gel is directly related to the amount of the serum released (Van den Berg *et al.*, 2008).

The gel breakdown properties were affected by the microstructure. These effects were evident in a test conducted by (van den Berg *et al.* 2007) where he compressed the gels, this leading to serum release that was directly related to the gels porosity. As compared to other gels, the high porous gels released a higher amount of serum. Large deformations and fracture properties of the gels were also affected by the serum release. The sensory perceptions of the gels was primarily governed by the breakdown system and serum release (Vandenberg *et al.* 2007).

Serum was expelled by the gels during deformation thus affecting the fracture properties and gels large deformations, states Van den Berg and his co-workers. The differences in the serum release were evident from the sensory differences conducted through the sensory analysis.

During large deformations, Van den Berg observed that varying amounts of serum was released from the gels with varying microstructures. The different mechanisms happening during the deformation of gels controlled the serum release, states Van den Berg. Darcy relations were used to explain the serum flow through a porous material. This relationship has been expressed as follows:

$$Q = \frac{BA_c}{\eta} \frac{\Delta p}{l} \quad (2.7)$$

where (Q) is the volume flow rate of serum permeating through the permeable area (A_c), (B) are the permeability coefficient, (Δp) is the pressure difference acting on the serum over a distance (l) and (η) is the viscosity of the liquid. The serum flow is affected by three factors as seen from the relation; stress, porosity and permeable area. The increase in the deformations led to an increase in the stress term. The serum expulsion is governed by the porosity, which is affected by the high release from the gels. The balance between the stresses needed for the serum flow will be determined by the serum flow. The permeable area will affect this balance and is associated with all the side walls of the gels (van den Berg et al. 2007).

The serum phase was compressed during the deformations, states van den Berg, which caused an immediate serum release. The pore size and the connectivity were reduced with an increase in the deformations. A lower area fraction of the pores was obtained due to a reduction in the pore size. In the presence of serum expulsion, the structural porosity will be reduced leading to a levelling off the serum release. During the deformations, the permeable area was also reduced. It can be assessed that almost all the serum is expelled from the gels sides as the top and bottom side of the cylindrical gel pieces come in contact with the compression plates. The gels porosity is related to the amount of serum released from the gel when compressed. This implies that a high amount of serum is released from the gels with high porosity. Large deformations and fracture properties of the gels are severely influenced by the serum release.

2.11.4. Release from Low Acyl Gellan Gels

When gellan gels are stored at ambient temperatures or under refrigerator temperatures, they will prove to be rather stable as compared to others. But at lower polymer concentrations, there might be little release of liquid (Gibson & Sanderson 1997). Thickeners are added in food products to avoid syneresis, which is not desired in foods.

When the gellan gels are squeezed, the release of fluid is observable (Gibson & Sanderson 1997) (Morris et al. 2012a). A study conducted by (Nakamura et al. 2001) with various compression rates showed these observations. There are two different mechanisms according to which the gellan gels will react to the compression; elastic deformations till the fracture point and rearrangement of the polymer network to incorporate the strains applied. The results concluded that the second mechanism became more dominant when the compression rate was reduced thus giving more time for rearranging.

As compared to the fluid products, the gelled product has lower perceived intensities of flavours and taste. A similar concentration of flavoured compounds such as salt and sugar were observed. Gellan has much better flavour release properties as it leads to less suppression than most other gelling agents. This may be owing to the release of water from the gel during mastication, explained (Morris et al. 2012b) (Gibson & Sanderson 1997), which also carried the flavour and taste compounds with it. Gellan was also compared to other gelling polysaccharides spanning a wide range of different textures through sensory and rheological comparisons states Gel rev, (Papageorgiou et al. 1994) (Ptitchkina et al. 1994), 1995. When water is

released it provides a mechanism to transport the flavour compounds across the fresh surface through fragmentation of the gel networks, which will explain the release of flavour accompanied with the expulsion of fluid under compression.

2.11.5. Interaction between Flavour Release and Gel Structure

The perception of gelled food products is also governed by flavour release besides texture. There is a perceived change in flavour with the addition of hydrocolloids to food. This mechanism still needs to be understood. There is a reduction in the effective concentration of proteins due to the binding of the protein flavour molecules with the protein. A comparatively small amount of binding can impose a significant influence with respect to the perceived flavour, as flavour is found in food at low levels. Food products with high water content will show a minimum binding of flavour to most other hydrocolloids (Renard et al. 2006).

The increase in the viscosity of the products containing hydrocolloids needs to be explained alternatively, if the binding of flavours to hydrocolloids is minimum. This situation implies that these products will have a reduced perception of flavour owing to a lower mass transfer and a slower release of flavour. The perceived intensity will be modified in the presence of hydrocolloids that will result in a modified flavour perception and a direct affect on the texture (Weel et al. 2002). According to Taylor et al., 2001, the gelatine concentration can influence the reduction of the intensity of furfuryl acetate (Taylor, 2002). The amount of flavour will remain unaffected by the texture of the gelled food containing the flavours, while the perception of flavour is changed (Renard et al. 2006).

The mixing between the thickener and the flavour molecules will be suppressed by the gel formation, which will in turn discourage the movement of flavour towards the taste buds. A greater obstruction in the taste perception is experienced in case of gels. (Clark 2002) reported the existence of an inverse relationship between taste intensity and hardness of soft solid foods. A negative relationship was observed between the overall perceived flavour and the gel hardness for the various types of biopolymer gels by (Clark 2002) when he conducted a comparative analysis among the various types of biopolymer gels containing 16% of sucrose. The sweetness perception tend to decrease with the increase in the polymer concentration, when (Gonzalez-Tomas et al. 2007) experimented with the k-carrageenan and gellan gum gels containing sucrose and aspartame. However, the previous findings have not been satisfied by these results. Flavour perception was negatively correlated to both the yield stress and yield strain, when a study was conducted with gels with a wide range of failure properties. The rating for perceived taste and flavours was extremely high for gels with equally perceived firmness but higher brittleness. According to conclusions, brittleness instead of hardness will define the taste and flavour perception in gels. The formation of fresh surface upon chewing accompanied the proposed mechanism for release of flavour molecules in these systems (Sala et al. 2010).

The microstructure of the gel primarily dominated the phenomenon of serum release. The highest amount of serum release upon compression was observed by (van den Berg et al. 2007) in case of gels with bicontinuous microstructure. This process can however be enhanced with serum release and develop the perception of tastants in

the gelled products, as flavour and tastants need to be dissolved in saliva before the taste buds can perceive them (Sala et al. 2010).

Gellan gum concentration, according to (Sala et al. 2010) determined the mechanical properties of the gellan gel, hence the increase in the gellan gum concentration led to an increase in the failure stress and failure strain. Sugar concentration will determine the stress and strain at the failure, which means that the addition of sugar led to an increase of the Young's modulus and a reduction in the failure stress and strain. The effects of gellan concentration on serum release were studied by (van den Berg et al. 2007). According to their observation, the addition of sugar led to a reduced release of the serum. In case of fructose, the sugar concentration was lower, while in case of sugar concentrations above 10 wt%, the effect was greater. Gellan gum and sugar concentrations also governed the serum release and the microstructure of gellan gels, states (van den Berg et al. 2007). There was an intense change in the microstructure of the gels owing to the increase in the gellan gum concentration.

During the compression tests, (Koliandris 2008a) observed that the brittle gels had more salts released as compared to the soft elastic gels. They observed an inverse relationship between the salt release and the failure strain.

2.11.6. Mechanical Properties of Gels

2.11.6.1. Force/Deformation Technique for Measuring Texture

One of the widely and commonly used methods to measure the textural properties of food is the Force/deformation technique. The mechanical properties that can affect

the sensory perception of the food can be measured individually or collectively with the help of this technique. Different methods of Force/deformation technique have been developed on the basis of the engineering theory of the materials. These methods are used to measure the distinct mechanical properties of food.

1) Destructive

2) Non-destructive

Among these two methods, the destructive methods are preferred for the measurement of the food texture. This preference is due to the reason that the destructive methods have a deeper relation to the sensory evaluation than the non-destructive methods (Bourne 2002). The primary destructive methods can be used to measure basic mechanical properties of the food such as the Young's modulus, failure strength, yield strength, failure stress and failure strain however the measured values of these variables cannot be determined by the human perception of texture.

2.11.6.2. Compression Test

Valuable information regarding the structure of materials can be obtained by the use of small deformation tests, which can then be used to predict the performance of the food in mouth and to classify food gels. Compression test is an important technique that helps to measure various mechanical properties of the food such as: the magnitude of rupture force, the basic deformation parameters such as Young's modulus of elasticity and the maximum bearable strain.

The textural analysis is one of the most commonly used methods for the characterization of food on mechanical grounds. The use of this method for the study

of gels has also been enlarged in the recent years (Coviello et al. 2005) (Jones et al. 1996).

The compression testing of the food is carried out in a universal testing machine with uniaxial loading. The food sample used for this purpose is cylindrical in shape. Fundamental mechanical properties of a large piece of food with a well defined shape can also be measured by the compression test. Two different methods of compression tests can be used for the testing of large pieces of foods.

- 1) The Uniaxial compression test of food samples between two plates;
- 2) The limited compression test such as extrusion;
- 3) The Uniaxial compression method employs the application of force to one side of the sample the other two sides of the sample are left to expand. Force is applied until the sample starts breaking or gets squeezed. The modulus of elasticity of the sample is the measured through the initial portion of the force/deformation curve;

Compression analysis experiments is among the exquisite techniques used for the measurement of textural properties of the food items like gels. A number of mechanical parameters of the food can be drawn from these experiments. Some of the typical parameters are:

- 1) Hardness of the gel (the maximum positive force needed to achieve a given deformation);
- 2) Strength of the gel (the stress that a specimen can sustain before failure);
- 3) Deformability (the amount of deformation or strain that a specimen can sustain before failure);

- 4) Brittleness (the tendency of a solid to fail suddenly after a very small deformation);
- 5) Module of elasticity (the ratio of stress to strain in linear region);

Compression tests have now become the primary methods for the measurement of the deformation properties of the food materials. The compression tests are easy to perform, the sampling is easy and do not have the gripping problems therefore they are preferred over tensile tests. The sample used here is normally cylindrical in shape having a diameter and height of 20 mm each. This sample is allowed to compress between the plates of a suitable machine, where the magnitude of the force exerted by the machine is the function of constant deformation rate. Stress (σ) is measured by dividing the force by the adjusted area and Strain (ϵ) is the relative change in the height or length of the sample ($\frac{dh}{h}$).

$$\epsilon = \int_{h_0}^h \frac{dh}{h} = \ln(h/h_0) \quad (2.8)$$

$$\sigma = Fh / \pi r_0^2 h_0 \quad (2.9)$$

The height and radius of the sample before and after the experiment are related if the volume remains constant.

$$\pi r_0^2 h_0 = \pi r^2 h \quad (2.10)$$

2.12. Scanning Electron Microscopy

2.12.1. Scanning Electron Microscopy (SEM)

The scanning electron microscope (SEM) utilizes a pointed beam of highly energized electrons to generate various signals at the exterior of the solid sample (Slayter, 1992). The high energized electrons have the ability to penetrate the surface of the sample to a small distance, and then the secondary electrons enter the penetrated surface and get attached to the positively charged detector and get amplified to produce a signal. The intense electron beam is then directed into a cathode ray tube (CRT). In the CRT the position and intensity of each beam is the representation of an equivalent spot on the surface of the sample used, hence a topographical response of the sample surface is obtained on the screen of the tube with a large field depth. The signal thus produced, varies according to the quantity of electrons and topography of the sample. These signals reveal the data about the sample that includes the texture or the morphology of the sample, chemical content, crystalline structure and lastly the orientation of the material that the sample is made up of.

The structure of the gel can be studied using SEM. The structure is generally studied using the rapid freeze etching method which has been found effective (Samichi & Ya, 1997) (Figure 2.6). In SEM, the image is produced point by point while the electron beam is focused across the surface of the sample. The initial penetration in the solid sample is done by the primary electrons which are then deflected due to a large number of elastic scattering processes. Detailed information along with two different contrasts is obtained with the help of the energy spectrum formed by the electron leaving the sample.

Mao *et al.* (2001) studied the water holding capacity of gellan gum affected by composition and microstructure. The co-existence of two different types of microstructures was observed by SEM. In the gellan gels, large and small pores were formed with thick strings of the network and a thin web structure respectively.

Gan *et al.* (2008) studied the physical properties of the microstructure of soy protein isolate gels. The gel is linked in the form of a network structure. The microstructures of soy protein isolate gels were studied using field emission scanning electron microscope (FESEM). The images showed that the gel revealed a sheet like structure with thick strands and large pores. The soy protein was in a three dimensional filamentous structure (Ornebro, Wahlgren, Eliasson, Fido, & Tatham, 1999). The Soy protein gels are of different dimensions and range from an ordered network strands to phase separated or aggregated structures, to an extreme where the protein is insoluble and a gel is formed (Hermansson, 1986).

Charoenrein *et al.* (2011) observed the microstructure of rice starch gel in SEM before and after 1 and 5 freeze–thaw cycles for the gels with and without Konjac glucomannan (KGM). All freeze–thaw starch gels developed a spongy structure which can be due to ice crystal formation and amylose retrogradation. A thick fibrillar network of starch gel was formed in the spongy structure during the repeated freeze–thaw cycles similar to Ferrero *et al.* (1993).

Picone and Cunha (2011) studied the microstructures of the gels at the different pH values using SEM. Overall the micrographs showed very compact structures with a innumerable pores, typical of gellan gum networks (de Jong & Van de Velde, 2007; Picone & Cunha, 2010; Yamamoto & Cunha, 2007). Acidification was seen to close

the pore network. The gels at pH7.0 showed a flat and closed network, while those at pH 5.3 were more continuous and with less pores. At pH 3.5 the network was more continuous and with smaller pores that were homogeneously distributed throughout the structure.

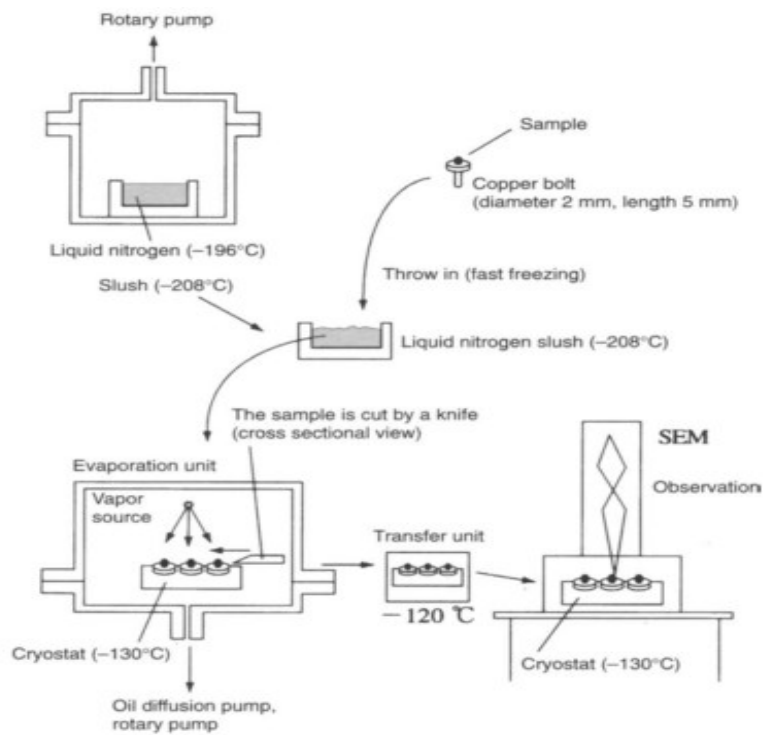


Figure 2.6 Fast Freezing Etching Method (Samichi & Ya 1997)

2.12.2. Cryo-Electron Microscopy

Cryo Electron Microscopy is a method used for the preservation of the hydrated biological specimen microstructure in vitreous ice. The first technique used for this purpose is the plunge-freezing (Adrian et al. 1984; Al-Amoudi et al. 2002; Al-Amoudi et al. 2004). This technique has continually been improved and modified according to the requirements of the tests (Klang et al. 2013). The use of cryogenic technique for the preservation and imaging of food specimen is gaining more recognition as an alternative to the old SEM techniques.

The small food specimen is frozen by dipping them into cyogen liquid in the Cryo-SEM technique. Minute crystals of ice are formed due to the rapid cooling of the specimen. These crystals are removed with the help of sublimation so that the structure of the specimen can be revealed. The thermal properties of propane make it a better cyogen for specimen with high moisture content. Propane provides a higher rate of freezing due to its low melting point, high boiling point, specific heat and good thermal conductivity (Robards & Sleytr 1985).

The use of Scanning electron microscopy has been employed on a large scale without Cryo preservation for the study of microstructure of food products like cheese. Although, this technique has a number of disadvantages (Fallico et al. 2006). The sample used for the observation in the Scanning electron microscopy has to be dehydrated properly as the microscope operates under high vacuum (10^{-4} Pa). If the water is not removed completely then it may vaporize in the microscope due to the low pressure. This increases the possibility of artefacts and may lead to change in microstructure observation, causing difficulty in the interpretation of data (Hassan

et al. 2003). These problems can be minimized by the use of Cryo scanning electron microscopy. That is why the use of cryogenic techniques is being widely accepted for the imaging and preservation of the food specimen, in comparison to the SEM. the Cryo SEM technique utilizes non-crystalline ice for the preservation of the sample. The tetrahedron of water is maintained in the ice slow cooling at temperature above -70 °C, at a pressure of 1 atm. This non crystalline ice gets linked to the hexagonal crystals to prevent the alteration in the microstructure sample (Kuo & Gunasekaran 2009). The formation of ice at atmospheric pressure may produce two other types of the ice i.e. the amorphous ice or the cubic ice. The amorphous ice also called the vitreous ice has a non-crystalline glass-like structure while the cubical ice is a warm type of the amorphous ice. The amorphous ice is produced as the sample is introduced in liquid nitrogen at -210°C for the preservation of the sample in Cryogenic observation (Kasas et al. 2003; Ong et al. 2011). The freeze fracturing can also occur in the specimen under the cryogenic preservation conditions, which may expose the inside microstructure of the sample. In this condition, water can be removed from the sample by Etching to unveil the internal structure in detail. Therefore, the use of cryogenic technique provides a better view of the internal structure of the specimen, in comparison to the dehydrating techniques, despite of the critical sample preparation procedure.

Mandelkow *et al.* (1991) studied the structural basis of dynamic instability we have examined growing, shrinking, and oscillating microtubules by time-resolved Cryo-SEM. The complete characterization of gel networks by mechanically through oscillatory and static rheology and optically through Cryo-SEM and turbidimetry. This shows the extensive structural change the neutralization of the polysaccharide by

bivalent calcium ions. This calcium ion is responsible for a marked aggregation of the polymer strands reminiscent of precipitation (MacArtain *et al.*, 2003).

Previous studies have reviewed the application of Cryo-SEM (Sargent, 1988) provide a valuable introduction to the aspects of food structure that can be revealed by these techniques. The main differences between conventional transmission electron microscopy and Cryo- electron microscopy of biological specimens arise from the requirements to keep the specimen below the devitrification temperature, to minimize contamination and electron dose, and to enhance the inherently low contrast in unstained specimens. In Cryo-electron microscopy, aqueous specimens can be examined by maintaining the devitrification temperature ($\sim -214^{\circ}\text{C}$) so as to prevent the conversion of water in to crystalline state. A numerous designs for liquid nitrogen-cooled, Cryo-specimen have been described by previous studies (Trujillo *et al.*, 2002; Błaszczak *et al.*, 2005 & Ying *et al.*, 2011). Therefore, the use of cryogenic technique provides a better view of the internal structure of the specimen, in comparison to the dehydrating techniques, despite of the critical sample preparation procedure.

Chapter 3. Materials and Methods

3.1. Introduction

This study aims to examine the effect of low acyl gellan gum and salt concentration on the mechanical and textural properties of gellan gel. Further examines the effect of these properties on the release from the gels based on the chapters 1 and 2. Moreover, this study includes the effect of changing the formulation of gellan gels and cyclic compression on salt and riboflavin release.

The material and methods used in this study is given as follows:

3.2. Materials

Some of the materials used in this study for carrying out the examinations such as:

- Low acyl gellan gum KELCOGEL from CP Kelco (Surrey, UK)
- Sodium Chloride NaCl from Merck (Hertfordshire, UK)
- Riboflavin (Vitamin B2) from Sigma-Aldrich (Poole, UK)

3.2.1. Gel Samples

The low acyl gellan gum (KELCOGEL) is a hydrocolloid derived from the microorganism *Sphingomonas elodea*. It is a water-soluble polysaccharide produced through fermentation. This gelling agent can be used alone or in combination with other products to produce a wide variety of distinguished textures. Commercial low

acyl gellan gum was produced by removing the acetate and glycerate groups using a strong alkali treatment (Huang *et al.*, 2004). The gel samples were carried out by using the commercially obtained food grade biopolymer of low acyl gellan gum, also called as 'Kelcogel' (CP Kelco). The obtained polysaccharide exists as a mixed salt and predominantly in the Potassium form but it also contains sodium or calcium salts (Nussinovitch, 1997). The formation of the powder gel was done by using the given manufacturer's manual. Various types of formulations were made for comparison Based on the percentage weight the concentrations are given in Table 3.1. All samples have been prepared in 100 ml of the solution.

Table 3.1 Different combination of gellan and salt concentration for gellan gel samples used in the experiments (% weight).

Gellan Concentration (%)	Salt Concentration (%)
0.6	0.5,0.6,0.7,0.8,1,1.2, 1.4,1.6,2,2.2,2.4,2.6
0.8	0.5,0.6,0.7,0.8,1,1.2, 1.4,1.6,2,2.2,2.4,2.6
1	0.5,0.6,0.7,0.8,1,1.2, 1.4,1.6,2,2.2,2.4,2.6
1.2	0.5,0.6,0.7,0.8,1,1.2, 1.4,1.6,2,2.2,2.4,2.6

CP Kelco produced the low acyl gellan gums and these gums were used in the experiment without any prospective calculations. Firstly, the weighted gellan powder was scattered in de-ionized water at 22°C. The solution was heated at 97-98°C for one minute for the arrangements of the material. Secondly, NaCl (Merck) was included in the mixture of gellan to form a gel. The mixture was stirred for one minute then transferred to the plastic tube of which the internal diameter is about 21mm to get a transparent mixture. The mixed gellan liquid was initiated to create a gel at 70 to 84°C. This temperature setting is higher when compared to the forming of the low acyl gellan gel at 25 to 70°C (Tang *et al.*, 1997). The gel was further cooled in cooling tubes by using running water at 15°C for 10 minutes. These tubes were then placed in refrigerator at 4°C overnight and then sliced and compression tested

3.2.2. Riboflavin

Riboflavin (Vitamin B2) has many potential benefits other than in the food industry. It is fluorescent under UV light, and their dilute solutions (0.015-0.025% w/w) can be used to detect leakages in the bioreactors. Sigma-Aldrich is a leading Life Science and High technology in (Poole, UK) tested the Riboflavin supports the verification of the concentration levels. The concentration of riboflavin used in all the experiments was 0.1 mg. The formation of this was done by dissolving the riboflavin accurate mass in the distilled water and stirring it at the room temperature. The containers of riboflavin were prepared in the container by covering it with aluminium foil. All samples have been prepared in 100 ml of the solution.

3.3. Methods

3.3.1. Equipment

Following equipments were used in this study for carrying out the materials testing:

- Uniaxial compression, Instron (Buckinghamshire, UK)
- Cryo Scanning Electron Microscopy (Cryo-SEM), Philips XL30 ESEM (Oregon, USA)
- Conductivity meter probe, Mettler Toledo (Columbus, USA)
- Spectrophotometer, Perkin Elmer Company Limited (Massachusetts, USA)

3.3.2. Compression Tests

An Instron universal testing machine (Instron, UK) equipped with a 10 KN load cell and Bluehill software was used to evaluate all low acyl gellan gum gels specimen by compression testing. The cylindrical specimens, having 21 mm in diameter and 20 mm in height, were compressed between two plates at a certain crosshead speeds. This machine has a diameter of 40 mm and is created by plunger at a fixed crosshead speed of 1 mm/sec up to 70% deformation. A tissue paper was placed in between the bottom of the sample and lower disc to avoid slippage of the samples to lateral direction and also to absorb the liquid which is expelled from the gels. Force versus deformation data were gathered by the software to obtain deformation data for the gels. At least five measurements were recorded for each gellan gels.

The measurement is calculated for the experiments at room temperature. The force deformation data measured the Hencky stress (σ_H) and strain (ε_H) with the help of the equations given below.

$$\sigma_H = F_{(t)} \frac{H_{(t)}}{H_0 A_0} \quad (3.1)$$

$$\varepsilon_H = \ln\left(\frac{H_{(t)}}{H_0}\right) \quad (3.2)$$

where,

$F_{(t)} \equiv$ normal force at time (t);

A_0 & $H_0 \equiv$ initial cross sectional area and height of the sample, respectively; and

$H_{(t)} \equiv$ height of the sample at time (t);

The greatest mark of strain-stress curve suggests and provides the point of failure. At least three duplications were employed for every gel.

3.3.3. Water Release

After the completion of compression and positioning on two layers of a tissue paper at room temperature, the release of water was established. An analytical balance is used to determine the weights of the gels and such weights were used as initial weights that are used after every compression release of water from the gel that was

detached by using tissue paper. The water release from the gels calculates the variance between the initial and final weights of gels. At least three duplications were employed for every gel.

3.3.4. Cyclic Compression Test

The mechanical properties of the gellan gels were investigated using cyclic compression. For this, the samples of gellan gel obtained were soft, medium and hard gels. To conduct cyclic compression test, the samples of cylindrical gellan gels were created and applied. The specific samples were compressed at 500 times at a fixed crosshead speed of 1 mm/sec, and the maximum load was noted up to 60% of the maximum force at the failure point of every gel. According to the earlier method of force deformation data, the Hencky strain and stress were measured. After every fifty cycles, the water release was measured by evaluating of the water loss from gels and positioning the gel on a tissue paper at specific room temperature. These calculated gel weights were used as initial weights and an analytical balance was used to weigh them. With the help of tissue paper, the weight of the gel was measured as the final weight of the specimen and water release form the gels was eliminated after every fifty cycles.

3.3.5. Salt Release

3.3.5.1. Salt Release Experiments

A square jacketed vessel with dimensions of 200 mm by 100 mm and 50 mm in height is employed for the experiment of salt release. It is placed beneath a 40mm

diameter Instron to identify and examine the effect of cyclic compression during release. A conductivity meter probe was attached with the system (Mettler Toledo, in lab 710 platinum 4-cell conductivity search) and overhead stirrer (25 mm diameter propeller type).

Every sample was examined to monitor the release of the NaCl from the gel structure to a proximate volume of water. The NaCl release from the gel structure was monitored using a conductivity probe in the central water form. The highest probable conductivity was measured at 940 $\mu\text{S}/\text{cm}$ based on the earlier calibration curve.

In the initial phase, before placing these gels into refrigerator, each and every gel was sliced into cylindrical shapes with a height of 20mm and diameter of 22mm. In order to have a synchronized environment, the vessel was filled with 200ml of distilled water and left to equilibrate at 25°C and stirred continuously at 100 rpm. Vessel used the conductivity probe to regulate and noticed at every 2 seconds. After 10 seconds of the initialization of data log, one gel specimen was placed through sandpaper which was put in the specimen to avoid from slipping and then the gel was included in the vessel. The experiments were completed thrice and carried out for 1 hour at 25°C. The number of elements that affects the real maximums is drifting probe and minor sample difference. The outcomes were measured in the form of a complete release fraction and were stabilized. To certify that only a minor influence is made to the value despite of salt inclusion to the samples and constancy of a long term conductivity interpretation, the regulated experiments was performed at 0%.

.Every sample was examined to monitor the release of the NaCl from the gel structure to a proximate volume of water. The NaCl release from the gel structure

was monitored using a conductivity probe in the central water form. The highest probable conductivity was measured at 940 $\mu\text{S}/\text{cm}$ based on the earlier calibration curve.

3.3.5.2. Measurement of Salt Release

The measurement of salt release into the water is done by measuring conductivity of the proximate volume of water and a normal curve and presented as:

$$\text{Salt release (\%)} = \frac{\text{released Salt}}{\text{total Salt}} \times 100$$

Where, the released salt was calculated from the salt concentration measured in the total solution volume and total salt was the amount loaded in each specimen or hydrogel. All experiments were done in triplicate.

The salt release was measured in online. To adjust the calculation of the conductivity to the concentration, the established concentration of liquid was used. This is presented in the figure given below (Figure 3.1).

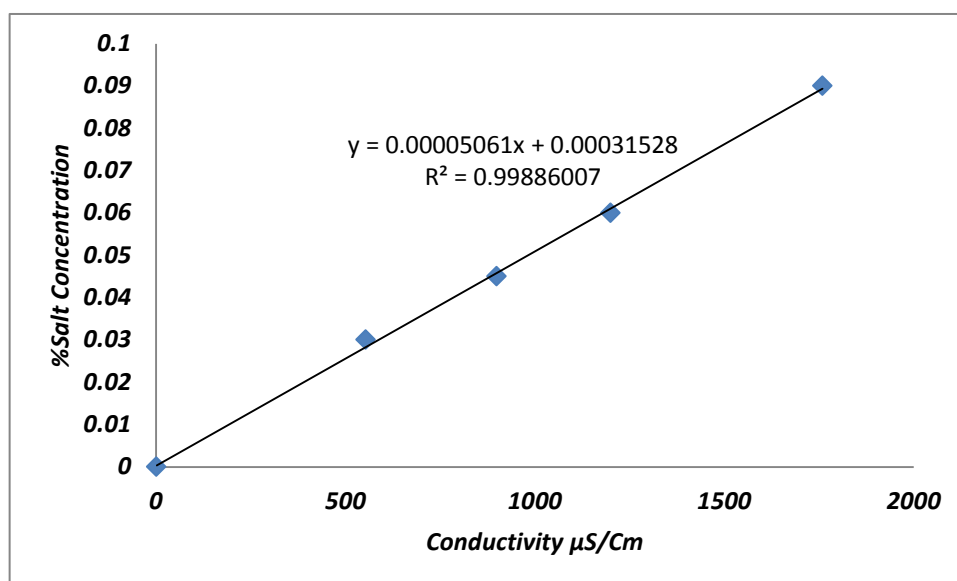


Figure 3.1 Calibration line of the known salt concentration versus the conductivity of the solution.

3.3.6. Release under Compression

This experiment focused on the second stage of experiments i.e. to assess the effects of the gel samples' cyclic compression on the release. By dropping the compression arm to the contact point, the samples were fastened with the vessel. The compressions were rolled for 10- 50 times constantly with 200 ml of water. It was rolled on for 10 times with 2 minutes' interval between every compression. As control, similar tests were conducted without compression.

3.3.7. Riboflavin Release

3.3.7.1. Fluorescence Spectroscopy

Fluorescence Spectroscopy is a process in which a molecule in an electronically excited state loses its electronic energy by emitting a photon. This spectroscopy was used in the present study to assess the fluorescence of riboflavin. The molecules of riboflavin attack the deepest vibration point of the ground electronic condition and when exposed to light, lift themselves up to reach the excited condition at room temperature. This exposition of light and molecules behaviour of changing themselves in either S1 or S2 condition is presented of changing themselves in either S1 or S2 condition is presented in Figure 3.2.

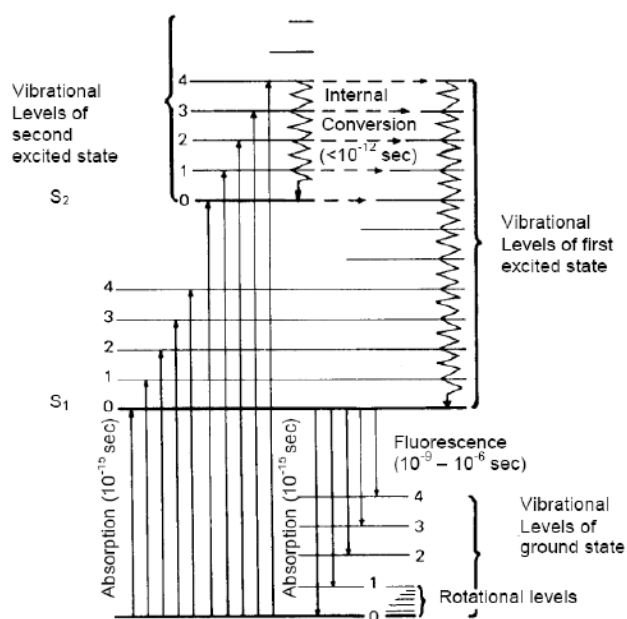


Figure 3.2 Transition giving rise to absorption and fluorescence emission spectra (Peerkin Elmer, 2006).

The change of molecules in excited state occurs when they touch any vibrational sub-points with every electron condition. The outcome should be in groups in a sequence of separate exposition as the energy is exposed in isolated amounts.

Molecules quickly lose their vibrational energy by hitting and dropping to the bottom stage of vibration from the excited state after the exposition of energy and touching the greatest vibrational levels of an excited condition. Molecules then produce their energy in fluorescence state and can go back to any vibrational condition of the bottom state (Elmer, 2006).

3.3.7.2. Equipment

Three standardized elements are presented in all fluorescence devices: a sample holder, a detector, and a light source Figure 3.3 . The wavelength of occurrence emission should be selectable and the signal of detector is able to specifically influence and offered in inclusion of analytic usage. To calculate of stable wavelengths in a standard filter fluorimeters, the wavelength of excited and released lights is chosen by filters. The spectral delivery of the light released from the sample that is fluorescence spectrum, can be detected by using standard fluorescence spectrometers in applying the two different methods like uninterrupted variable interference filter or a monochromator. The selection of exciting light and the examination of sample release can be done by monochromator in more state-of-the-art devices. These devices can be used to calculate the difference of release concentration with exciting wavelength and the fluorescence exciting spectrum (Elmer, 2006).

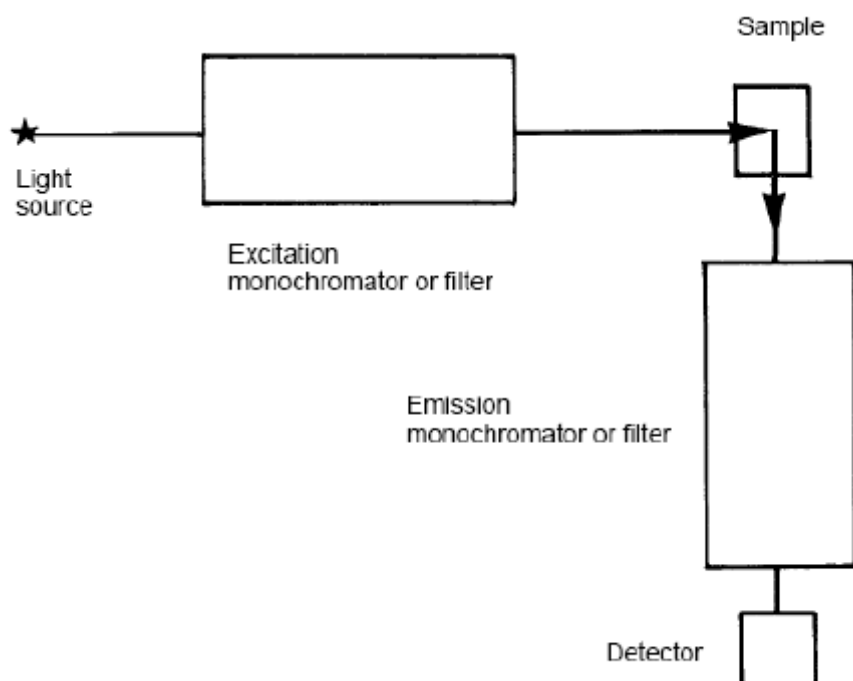


Figure 3.3 Essential components of a fluorescence spectrometer (Perkin Elmer, 2006).

3.3.7.3. Measurement of Riboflavin Release

Riboflavin released into the water was determined from the measurement of absorbance at 445 nm and a standard curve and it was expressed by the below formula:

$$\text{Riboflavin release (\%)} = \frac{\text{released Riboflavin}}{\text{total Riboflavin}} \times 100$$

where released riboflavin was calculated from the riboflavin concentration (mol/L) measured in the total solution volume and total riboflavin was the amount loaded in each specimen or hydrogel. To avoid photodecomposition of riboflavin, all experiments were performed thrice in the Amber vessel.

A 1.5 ml of sample was positioned in a cuvette for calculating the riboflavin concentration and its intensity was calculated by using the fluorimeters. Figure 3.4, depicts the standard curve between the intensity and the concentrations used. This graph will help in calculating the utilizing identified liquid concentration

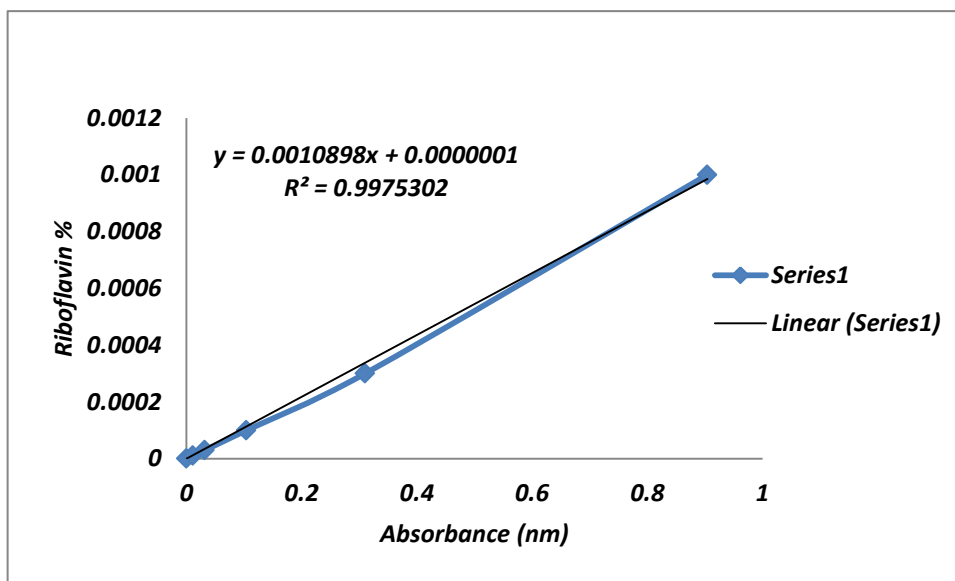


Figure 3.4 Calibration line of the known riboflavin concentration versus the intensity (absorbance) for riboflavin at 488 nm.

3.3.8. Cryo Scanning Electron Microscopy (Cryo SEM)

An important method is used to study the structure of gel is the Cryo-SEM. This SEM assesses and pictures the gellan gels specimen microstructure.

There are three basic sample creation systems for the Cryo such as a vacuum transfer device (VTD), a slushing station and sample creation chamber. The creation of gel for Cryo SEM was done by attaching on a copper holder. The VTD attaches itself with the copper holder that is fixed straight to the slushing chamber. At -210°C , the samples were submerged into liquid nitrogen slush (a mixture of solid and liquid nitrogen). To avoid the existence of particulates that may offer nucleation for the

development of ice crystals, the liquid nitrogen was immediately poured. It was poured into a polystyrene cup that was positioned in a slushing chamber, to produce the nitrogen slush. With the help of a rotary pump unit, the chamber was emptied by losing latent heat of vaporization; the nitrogen stopped to boil and set. The vacuum was emitted to permit the settled nitrogen is to dissolve after 30 sec. To ensure the freezing of nitrogen at -210°C and it is ready to use, the experiment was done twice. For 15 sec, the samples were submerged into immediately created slush.

With the help of the VTD into a fixed Cryo creation chamber, the frozen specimens were quickly replaced following its freezing. To avoid the ice recrystallisation that is the development of smaller ice crystals to a large size, the VTD permits the samples to place under vacuum ($>10^{-4}$ Pa) to the sample creation chamber and it results in minimizing the variation in microstructure. At -140°C , under great vacuum state ($>10^{-4}$ Pa), the sample was torn with the help of a chilled scalpel blade in the chamber with the help of an exterior binocular microscope. For 60 sec, the specimen was fixed (removing the ice from the fractured sample surface by vacuum sublimation) at -95°C for 20 min and covered with the help of cold magnetron sputter coater with 300 V, 10 mA of sputtered gold. The nitrogen gas cooled module takes it under vacuum at -140°C and follows utilizing a field release gun SEM at 5.0 KV.

The sample used for this purpose is normally dried and provided with electrical conductivity on the surface by the evaporation of gold or other material. The sample is then observed in vacuum at a pressure of around 10^{-3} Pa. Although, most of the gels have been used in wet conditions for the purpose of avoiding the structural damage due to provide precise measurements. For these purpose, a new technique

such as rapid freeze etching method, which are considered as more effective and it is employed in this study (Samichi & Ya 1997).

The use of solvent for the removal of hydrogel polymer can cause structural changes and shrinkage to the sample gel. This is because the use of water is desirable during the observation of the internal structure of the sample. The formation of the microcrystal can be covered up and the amorphous vitrification can take place by the rapid freezing technique. The required cooling rate was necessary for the amorphous vitrification of red blood cells is 3000 K/min, for yeast it is 10 K/min, and for a colon bacillus the required cooling rate is 6 K/min. The sample that requires a higher cooling rate than 104 K/min is dropped in liquid nitrogen for rapid freezing. The liquid nitrogen is then removed with the help of rotary pump and it is evaporated and the temperature is decreased due to the removal of heat of vaporization. Hence a sorbet-like slush (-208 °C) is obtained.

Minimal amount of bubbles are achieved and a good frozen sample is obtained by adding the liquid nitrogen. A high rate of cooling is achieved in this process, which is estimated to be more than 104 K/min (Samichi & Ya, 1997). This rapid frozen sample is then moved to a pre-cooled cryostage at a temperature of around -120 °C to -130 °C in vacuum evaporation equipment and then it is evacuated. The sample is then cross-sectioned with a knife to make it ready for observation. At this stage if the temperature raises to -80 °C then the water content present in the sample will not convert to liquid form, sublimation may occur and the water will be evaporated. Hence, there was no structural damage was observed. . If the level of ice gets low, freeze etching will occur, leaving only the polymer networks behind (Figure he T .(2.6

temperature of the SEM stage is maintained below -120 °C to preserve the structure of the sample during observation. This is why it is called Cryo-SEM (Samichi & Ya 1997).

Chapter 4. Mechanical Properties of the Low Acyl Gellan Gels and the Effect of the Cyclic Compression on Mechanical Properties of the Gels

4.1. Introduction

The important objective of the present study is to analyse the mechanical properties of gellan gels with the effect of salt and low acyl gellan gum concentration. In the literature review chapter, the deep study was carried out and explained biopolymer gels mechanical properties like gellan gum. However the effect of the cyclic compression on mechanical properties of the biopolymer gels has not been studied. The mechanical properties will be studied in this chapter:

- i. The effect of the cross head speed of compression on mechanical properties of the gel;
- ii. The effect of submersion of the gels in water on the mechanical properties of the gel;
- iii. The effect of the low acyl gellan gum concentration on mechanical properties of the gel;
- iv. The effect of the salt (cation) concentration on the mechanical properties of the gel;
- v. The effect of the cyclic compression on the geometry of the gellan gel specimens;
- vi. The effect of the cyclic compression on the mechanical properties of the gellan gels;

vii. The effect of the cyclic compression on the water release from the gels.

This knowledge and understanding will be important for analysis the flavour release from the low acyl gellan gum gels and the microstructure of the gels (Chapter 5 and 6).

4.2. Compression Tests

An Instron universal testing machine (Instron, UK) equipped with a 10 KN load cell and Bluehill software was used to evaluate all low acyl gellan gum gels specimen by compression testing. The cylindrical specimens, having 21 mm in diameter and 20 mm in height, were compressed between two plates at a certain crosshead speeds. A tissue paper was placed in between the bottom of the sample and lower disc to avoid slippage of the samples to lateral direction and also to absorb the liquid which is expelled from the gels. Force versus deformation data were gathered by the software to obtain deformation data for the gels. At least five measurements were recorded for each gellan gels.

4.2.1. Effect of Crosshead Speed of the Compression Test on Mechanical Properties of the Gellan Gels

To investigate the effect of the crosshead speed of compression test on the mechanical properties of the low acyl gellan gels like true stress, true strain, Young's modulus and energy per unit volume, compression tests were performed. By examining the force displacement curve acquired at different crosshead speed, the mechanical properties at failure point were collected. The lower and upper discs were placed at opposite to compress the gels at different crosshead speeds. The speed of crosshead was 0.5, 1 and 1.5 mm/sec.

Figure 4.2 shows the true stress versus true strain curve taken at different crosshead speeds.

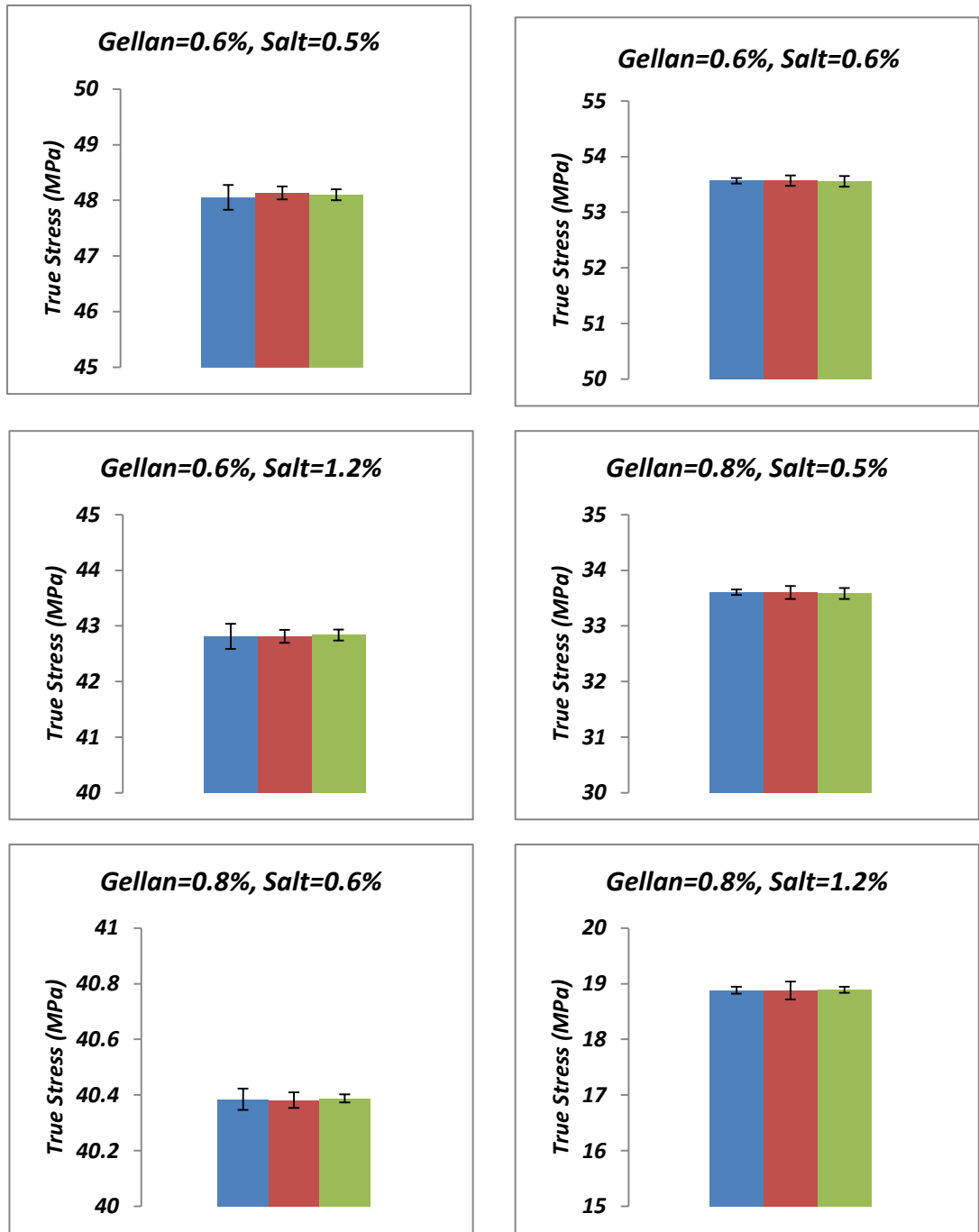


Figure 4.1.a True stress at failure point of the low acyl gellan gels at various crosshead speeds. The error bars show standard deviation from compression of three replicates.

■ Crosshead speed=0.5 mm/Sec
 ■ Crosshead Speed=1 mm/Sec
 ■ Crosshead Speed=1.5 mm/Sec

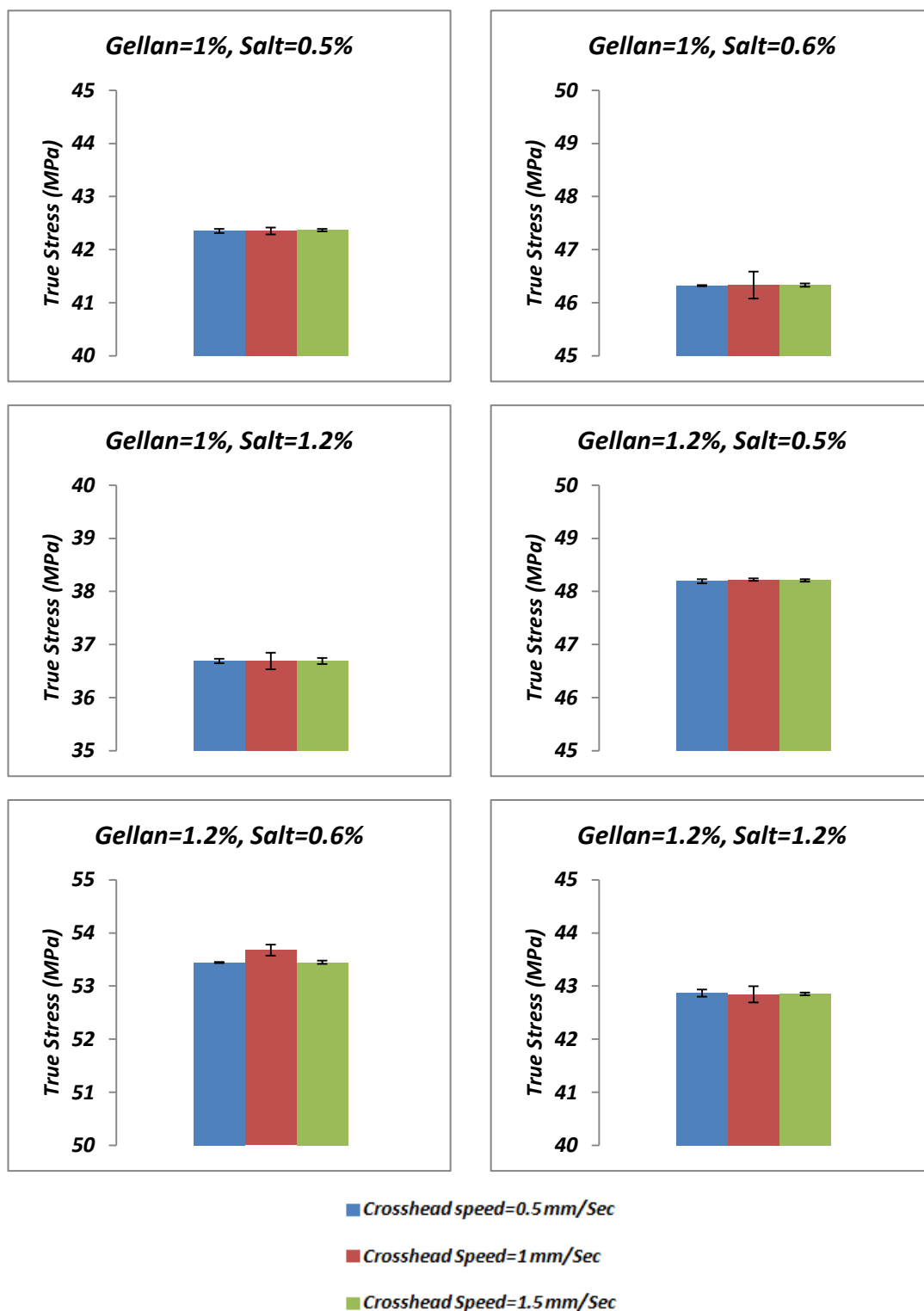


Figure 4.2.b True stress at failure point of the low acyl gellan gels at various crosshead speeds. The error bars show standard deviation from compression of three replicates.

Figure 4.2 shows that there is not much of difference between the true stress and true strain of the gels while subjected to different compression crosshead speeds; therefore, it can be concluded that crosshead speed in this range does not have any effect on the mechanical properties of the low acyl gellan gels. Hence, 1 mm/sec is selected as the compression crosshead speed for further compression tests. In previous studies cross headed speed of compression in the combination of high and low gellan gels were showed a similar findings (Huang *et al.*, 2003). In contrary to this, the mixed gels exhibited effect on their texture properties (Mao et al., 2003).

4.2.2. Comparison between Mechanical Properties of the Dry Gels and Immersed Gels in Distilled Water

In order to examine the mechanical properties of the dry gels and immersed gels in distilled water were compared.

The gels first detached from plastic cylinders then they split into cylinders of 20 mm height and 21 mm diameter and then put into an Instron universal testing machine. The testing machine uniaxially compresses the samples. To avoid the slippage of the gel, a tissue paper was put between sample cover and the lower disc of the Instron. Up to failure point, the gels were compressed at 1 mm/sec.

The true stress and true stain curves can provide the mechanical properties at failure point. Experiments were performed thrice by using various low acyl gellan concentration gels.

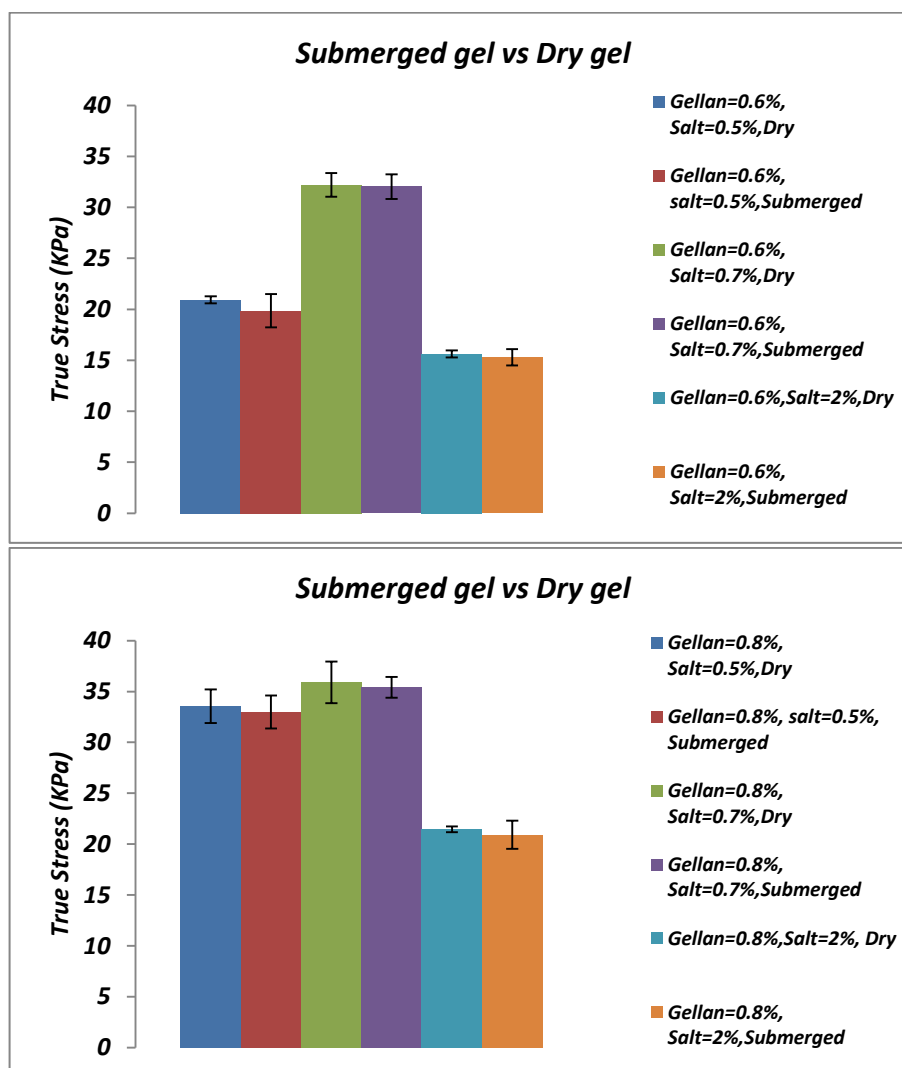


Figure 4.3 True stress versus true strain of submerged gels and dry gels. The error bars show standard deviation from compression of 3 replicates.

In Figure 4.3, it can be seen that there is no any significant difference between mechanical properties of immersed and dry gels; so, the immersing of the gels in the water doesn't have any effect on the gellan gels mechanical properties. The findings of the study is in line with previous study were the behavior of the swollen gellan gels showed that there is no change in mechanical properties during swelling in the combination of high and low acyl gellan gel (Silva *et al.*, 2013). In contrary to this

argument, the mechanically reinforcing gels have showed significant of their mechanical properties (Kirchmajer, 2013)

4.2.3. Effect of Low Acyl Gellan Gum Concentration and Salt Concentration on the Mechanical Properties of the Gels

To examine the effect of low acyl gellan gum concentration and salt concentration on the mechanical properties of the gellan gels, the uniaxial compression test was performed. At failure point, the limits of gellan gum concentration were employed to examine the effect of the gellan gum concentration on the mechanical properties of the gels. The variance of gellan gum was from 0.6 to 1.2% (w/w); in addition, the sodium chloride as a cation (cross linking agent) ranges from 0.5 to 2.6% (w/w). At a fix crosshead speed of 1 mm/sec, the gels were compressed up to the failure point the calculation of Hencky stress (σ_H) and Hencky strain (ϵ_H) from the force-determination data used the following equations.

$$\sigma_H = F_{(t)} \frac{H_{(t)}}{H_0 A_0} \quad (4.1)$$

$$\epsilon_H = \ln\left(\frac{H_{(t)}}{H_0}\right) \quad (4.2)$$

where,

$F_{(t)}$ = the force at time t ;

A_0 & H_0 = the initial area and height of the sample, respectively; and

$H_{(t)}$ = the height at time t ;

The maximum of stress-strain curve measures the failure point. Repetition was done for at-least five times for every gel. The mean of the maximum stresses and strains was taken while compression tests in five repetition gellan specimens were used for exposing the strength and deformability of the gellan gel samples. Figure 4.4

presents the effects of salt and gellan concentrations on true stress and true strain on the samples of the gellan gels at failure point.

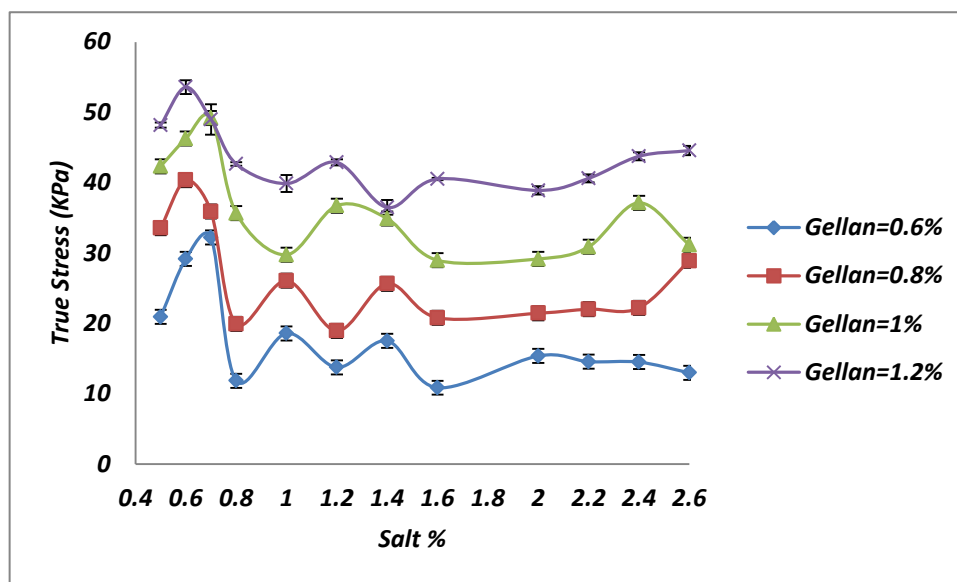


Figure 4.4 True stress as a function of salt concentration at different gellan concentrations. The error bars show standard deviation from compression of 5 replicates.

Stress at the failure point reflects the strength of the gel. It also represents the maximum external force that can be tolerated by the gel without fracturing, and the corresponding strain is an indication of gel deformability. The strength of the gellan gels increases with gellan concentration at all levels of salt (see Figure 4.4). It happens because of the higher concentration of the gellan that results in higher cross link densities, in the gel network; therefore, it forms stronger gels. Thus the gel strength in gellan gels decreased when cation concentrations were above the critical value (Sanderson & Harris, 1990; Tang *et al.*, 1994; Tang *et al.*, 1996).

If the salt concentration is more than 0.6%, or 0.7% weight (critical salt concentration), then the strength of the gellan gels sharply decreases with the increase of salt concentrations. Also, gellan gels are weakened by additional added

salt (Tang et al., 1996). It is in regard of the general agreement with the findings of (Huang et al. 2003). This might be due to the fact that when the cation replaces the anion in gellan gels, the molecules restricts cross linking of adjoined polymer chains and form weak structure (Tang *et al.*, 1994; Lau *et al.*, 2000).

Gellan gels are deformed at a relatively large extent in low salt concentrations. When the salt concentrations were more than 0.7% weight, true strain at the failure point increased sharply with the increasing salt concentrations (Figure 4.5). The true strain at the failure point attained to a maximum value. It is crucial to notify that the points where the strains approached maximum value in Figure 4.5 match closely to the salt concentration at the minimum strength of each gel in Figure 4.4. The samples with lower failure strains represented the gel exhibit a fracture easily. This finding is in line with the earlier study when the concentration of cation increases the gel tends to be more brittle. Thus the cation concentration plays a major role in making a gel brittle (Lau *et al.*, 2000). Similarly, the addition of cation ions increases the gel become more brittle was observed by (Hill et al., 1998; Rakde et al., 2015).

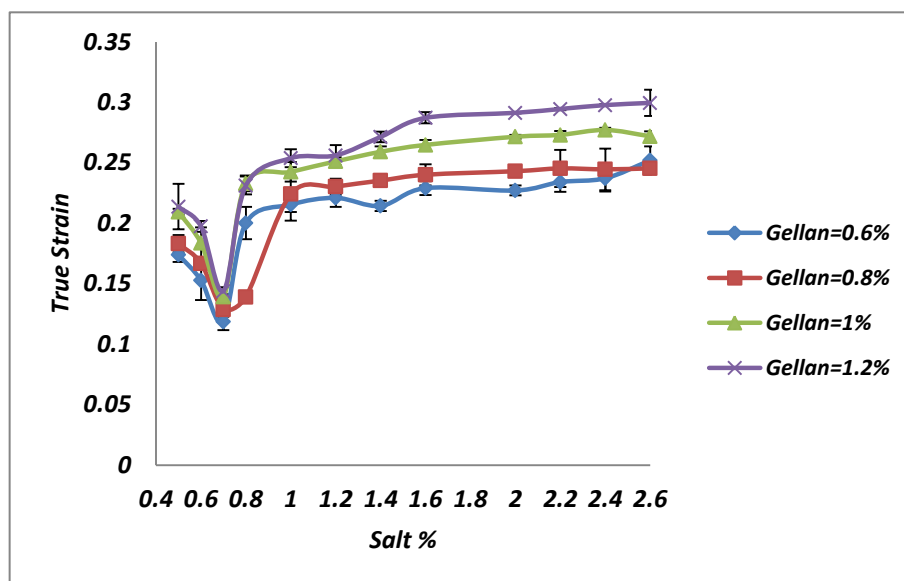


Figure 4.5 True strain as a function of salt concentration at different gellan concentrations. The error bars show standard deviation from compression of 5 replicates.

Since the Young's modulus is an indication of firmness of the gels, it can be said that other researcher's work recorded low acyl gellan gels with the increase in cation concentration; Young's modulus was initially increased and eventually decreased for reaching a constant value. Nonetheless, the failure stress of the low acyl gellan gels follows a similar trend as that of Young's modulus as a function of cation concentration, with regard to the findings of (Mao et al., 2000; Huang et al., 2003; Tang et al. 2004; Cuadros., n.d). It can be seen in Figure 4.6 that the Young's modulus is increased with the increase in salt concentration and reaches the peak at 0.7 weight % salt concentration. Then, it decreases to a constant value. Young's modulus reached a constant value at 0.8 weight % salt concentration, which was related to the observation of failure true stress that reached a constant value of 0.8 weight % salt.

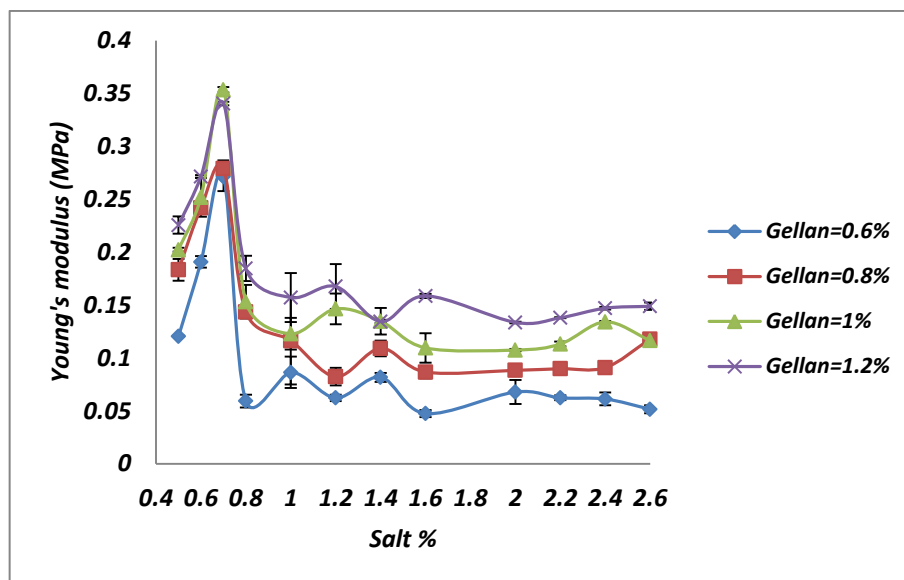


Figure 4.6 Young's modulus as a function of salt concentration at different gellan concentrations. The error bars show standard deviation from compression of 5 replicates.

As mentioned in section 2.7.5 the area under force-displacement curve during compression up to failure point can be transformed into the real amount of work which is needed to rupture the gel. The force applied during the compression can be used for indicating the rupture force or rupture strength of a gel. The areas under the force-displacement curve, which can be obtained from compression test, represents the amount of energy needed to breakdown and rupture the sample (energy per unit volume). The failure or breakdown of a gel is not only related to the strength, but it is also related to the energy that is required to rupture. A gel with extensive strength and less required energy per unit volume is destroyed or fractured more easily than a gel with lesser strength and high energy required per unit volume. Thus, by increasing gellan concentrations, energy per unit volume also follows an ascending order, that causes the gel not to rupture easily. By increasing salt concentration

above 0.6 weight %, the energy per unit volume has been sharply decreased; thus, the gels were ruptured more easily. As it can be seen in Figure 4.7, the energy per unit volume was comparable to the trend observed in failure stress.

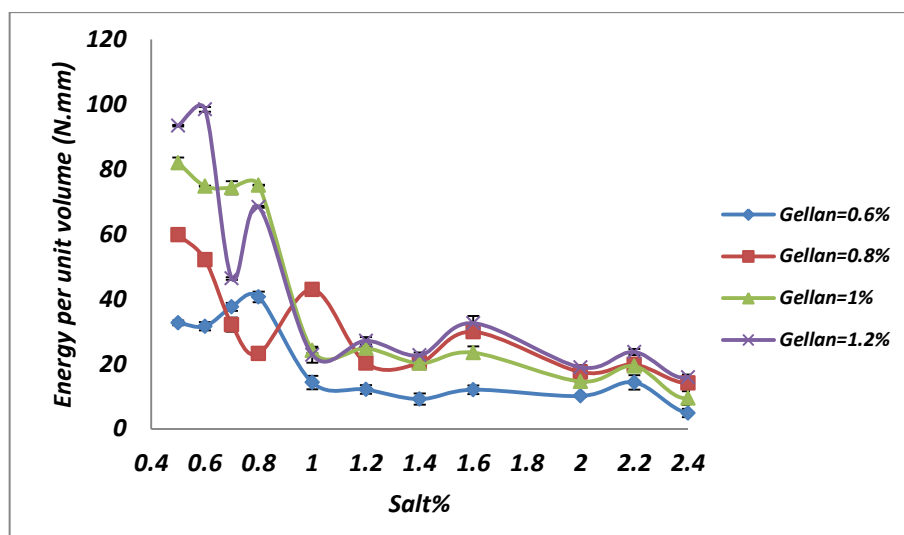


Figure 4.7 Energy per unit volume as a function of salt concentration at different gellan concentrations. The error bars show standard deviation from compression of 5 replicates.

4.3. Cyclic Compression Test

For the purpose of this study, samples of gellan gum gel were taken for covering a variety of gel strength, that is, soft, medium and hard gels. The samples of cylindrical gellan gel were prepared for the purpose of conducting cyclic compression tests by using the same procedure, as explained in the single compression section. These samples were compressed up to five hundred times at a fixed cross head speed of 1 mm/sec, and the maximum load was up to 70% of the maximum force at the failure point of each gel. The Hencky stress and strain were calculated according to the formula, as discussed in the previous section from the force-deformation data. Water

release was determined by weighing the water loss from gels when they were placed on a tissue paper at the room temperature after each fifty cycles. These gels were weighed with an analytical balance, and these weights were used as the initial weights. After each fifty cycles, water release from the gels was removed by tissue paper and the weight of the gel was determined as the final weight of the specimen. For each gel, minimum of three replications were carried out.

4.3.1. Effect of the Cyclic Compression on Geometry of the Gellan Gel Specimens

Sanderson et al, 1997, stated that while the gellan gels are under compression force and they are squeezing, it can be caused to water release from the gels structure. The shape of the gellan gels (21 mm diameter and 20 mm height) which was prepared with a concentration mix of 0.6, 0.8, 1 and 1.2% weight and different salt concentration, at 1 mm/sec crosshead speed, was compressed at cyclic compression ranging from 50 to 500 cycles. Figure 4.8 shows photographs of the gels taken immediately after cyclic compression at representative rates within the ranges (50, 100, 300 and 500 cycles).

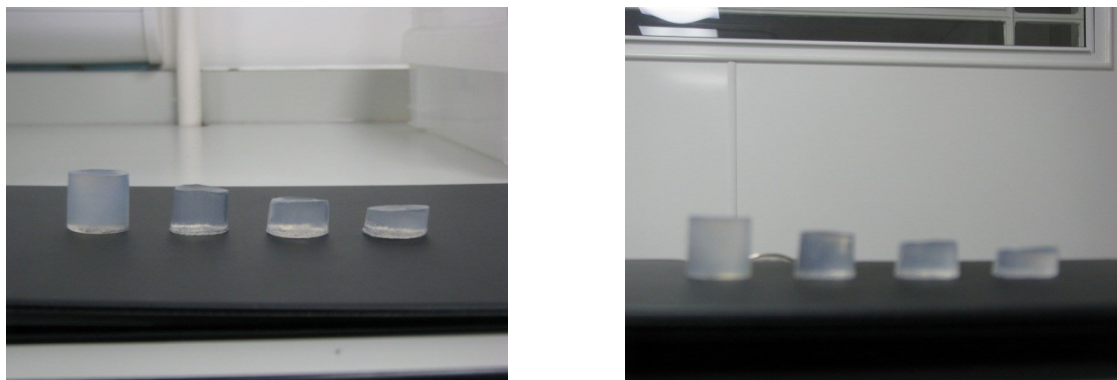


Figure 4.8 Low acyl gellan gels after 50, 100, 300 and 500 cyclic compressions respectively at 1 mm/sec crosshead speed.

As it can be observed in Figure 4.8 the geometrical composition of the samples was altered because of the water released from the gels as a consequence to the force applied on it during the cyclic compressions. The height of the specimen was reduced to half after the pressure exerted from 500 cycles, but the diameter has shown minimal changes in it, even after all this compression. This change in the dimensions can be attributed to the fact that water went out of these gels during the cyclic compressions and resulted in the height of the samples is decreased. Thus leads to the compaction of the linked network which enables the stress applied to distributed uniformly which leads to the gels with higher concentration of have higher failure strain or deformability (Yamamoto & Cunha, 2007).

4.3.2. Water Release During Cyclic Compressions

. The samples of cylindrical gellan gels were prepared for the purpose of conducting cyclic compression tests. Three gellan gels were selected as soft, medium and hard specimen for investigating the water release during cyclic compression from the gellan gels for each range of the low acyl gellan gum concentrations. These samples

were compressed up to five hundred times at a fixed cross head speed of 1 mm/sec, and the maximum load was up to 60% of the maximum force at the failure point of each gel. Water release was determined by weighing the water loss from gels when they were placed on a tissue paper at the room temperature after each 50, 100, 300 and 500 cycles. These gels were weighed with an analytical balance, and these weights were used as the initial weights. After each 50, 100, 300 and 500 cycles, water release from the gels was removed by tissue paper and the weight of the gel was determined as the final weight of the specimen.

The results of the water release percent, reduced height percent and reduced diameter percent following the cyclic compression test have been plotted versus the number of cycles. According to the obtained results the minimum and maximum water release after 500 cycles was 27% and 55%, respectively, also the reduced height percent after 500 cycles was 4 to 56 percent and the diameter reduced percent was 0.09 to 3 percent. The findings of the present study is in line with the previous studies where the higher the gellan concentration, higher the water holding capacity and provide a solid gel like appearance. Thus more resistant was observed in gellan gels with low gellan (Banerjee & Bhattacharya, 2011).

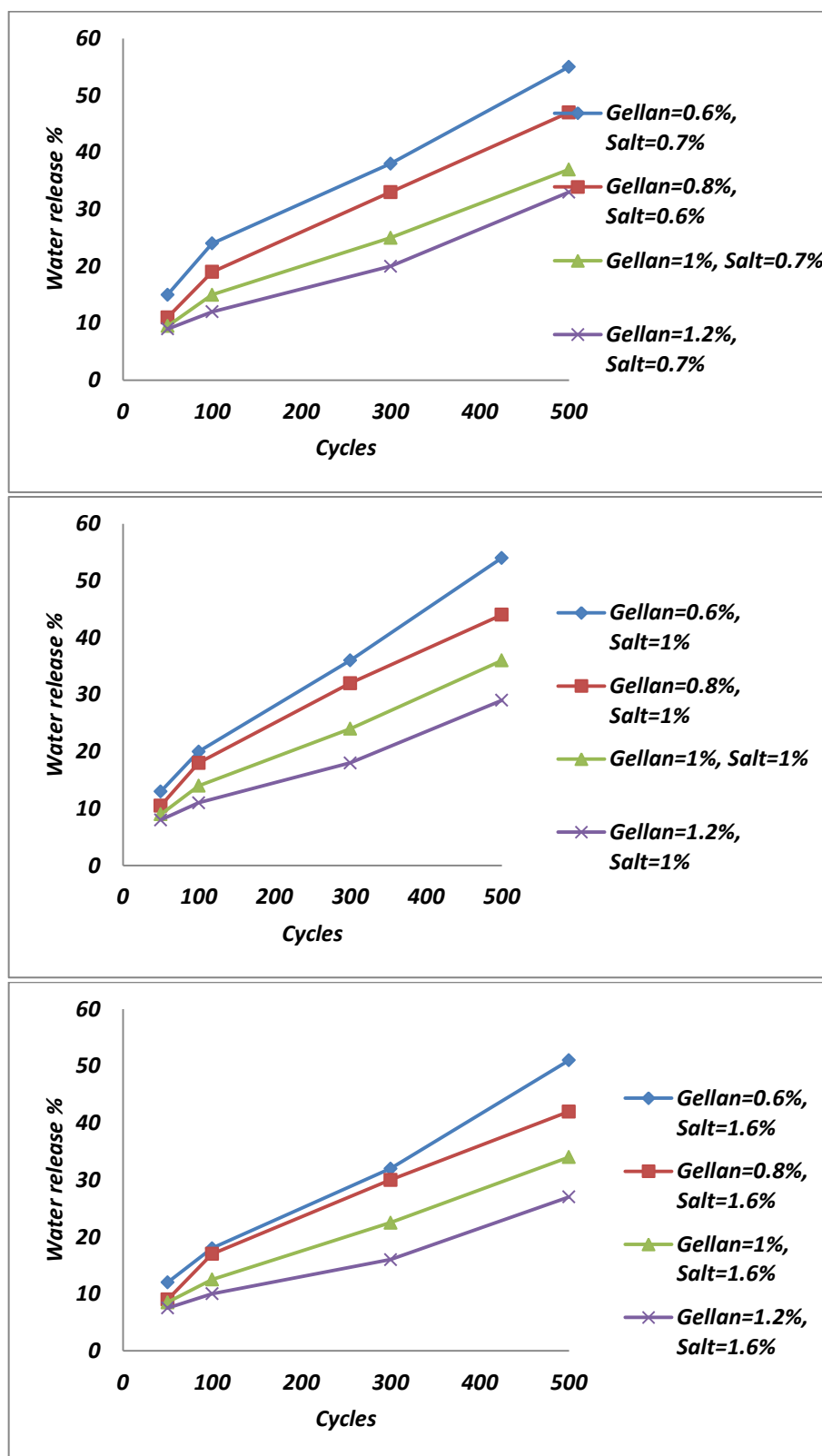


Figure 4.9 Water release percent from the gellan specimens during cyclic compressions versus number of cycles.

As it can be observed in Figure 4.9, water release is affected by salt concentration and gellan concentration. As these graphs show the water release is decreased by increasing gellan concentration, so it seems higher concentration of the gellan that results in higher cross link densities in the gellan network so amount of the water which is trapped in gellan network is increased, thus, the water holding capacity of the gel is improved, then, the water release is reduced.

The water release is affected by salt concentration, as the above graph shows by increasing salt concentration the water release is decreased. It can be assumed while salt concentration is increased (above the critical salt concentration) the excess cations result in higher hydrogen binding in the network therefore the ability of the network to entrap the water in the gels is increased, thus, water release is decreased.

The maximum and minimum reduced height percent after 500 cycles was 54 and 29 percent, respectively. As the graphs in Figure 4.10 show by increasing gellan concentration the reduced height percent is decreased, it can be concluded that above critical salt concentration by increasing salt concentration the water release is decreased thus the volume change due to little change in diameter of the samples is decreased. By increasing gellan concentration reduced height percent is decreased and due to little change in diameter of the samples the volume change of the samples are decreased.

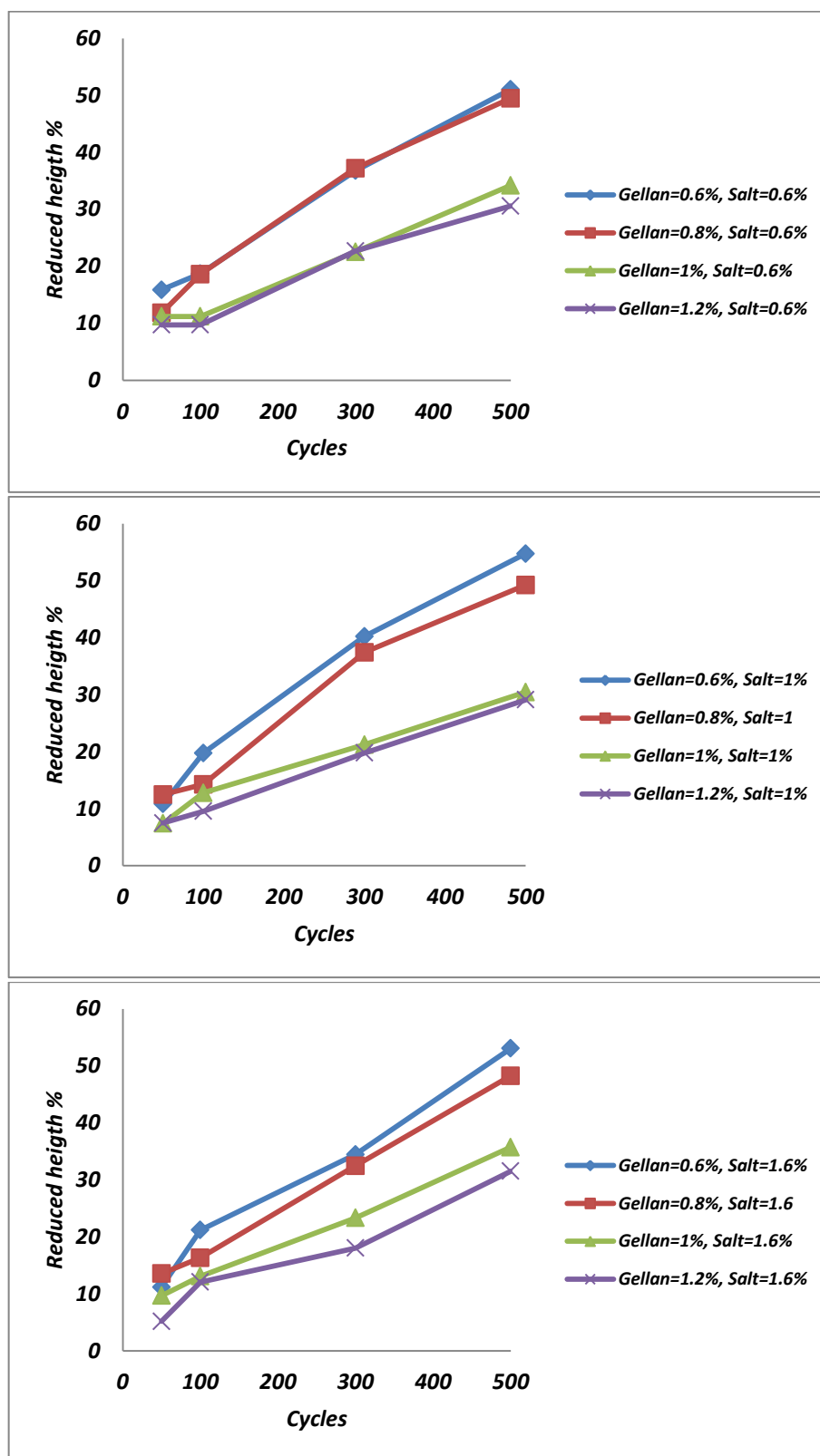


Figure 4.10 Reduced of height percent of the gellan specimens during cyclic compressions versus number of cycles.

In Figure 4.11, the most change in the diameter of the cylindrical gels which were used as samples is 2.32 which in comparison to the change in height of the structures are negligible. Hence, the major change in volume of the structures is only attributed to the change in height of the cylindrical gels. Thus when the degree of hydrocolloid increased, there is a increased amount of water to form solid like gel, hence it is resist more than the low-level hydrocolloid gel (Banerjee & Bhattacharya, 2011).

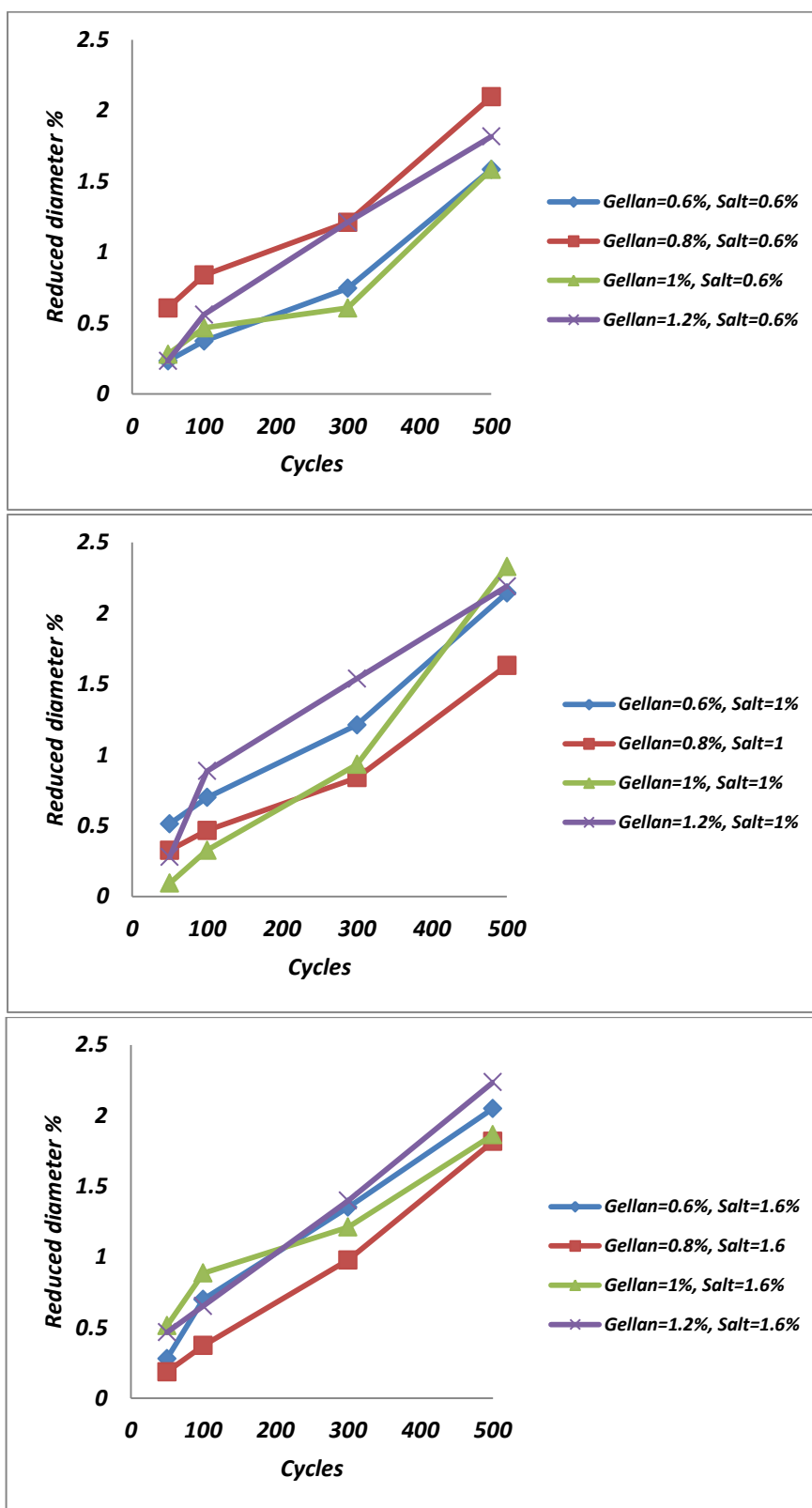


Figure 4.11 Reduction of diameter percent of the gellan specimens during cyclic compressions versus number of cycles.

4.3.3. Effect of the Cyclic Compression on the Mechanical Properties of the Gellan Gels

The cyclic compression was carried out on the gellan gels as a precondition, and then the gels were compressed until failure. Three gellan gels were selected as soft, medium and hard specimen for investigating the effect of cyclic compression on mechanical properties of the gellan gels for each range of the low acyl gellan gum concentrations. Five hundred cycles were set up on cyclic compression test, and the maximum load was up to 60% of the maximum force at the failure point of each gel. The height of the samples was reduced to 50% of initial height after 500 cycles. Further, the samples lost plenty amount of water during cyclic compressions test. It was observed that by reducing the height of the samples, and water release during cyclic compressions, mechanical properties of the gels were changed at the end of cyclic compression tests.

Figure 4.12 shows the effect of the cyclic compression on true stress versus true strain curve as it can be seen that true stress is decreasing during the cyclic compression as cyclic compression resulted to release water from the gels. Thus, it seems that by reducing the water content in the gels, the structure of the gellan gels was changed, and it can be assumed that a new gel with more gellan concentration and lower water content was created. Therefore, the strength of the gels was grown up, the true strains were decreased, and the energy per unit volume was increased.

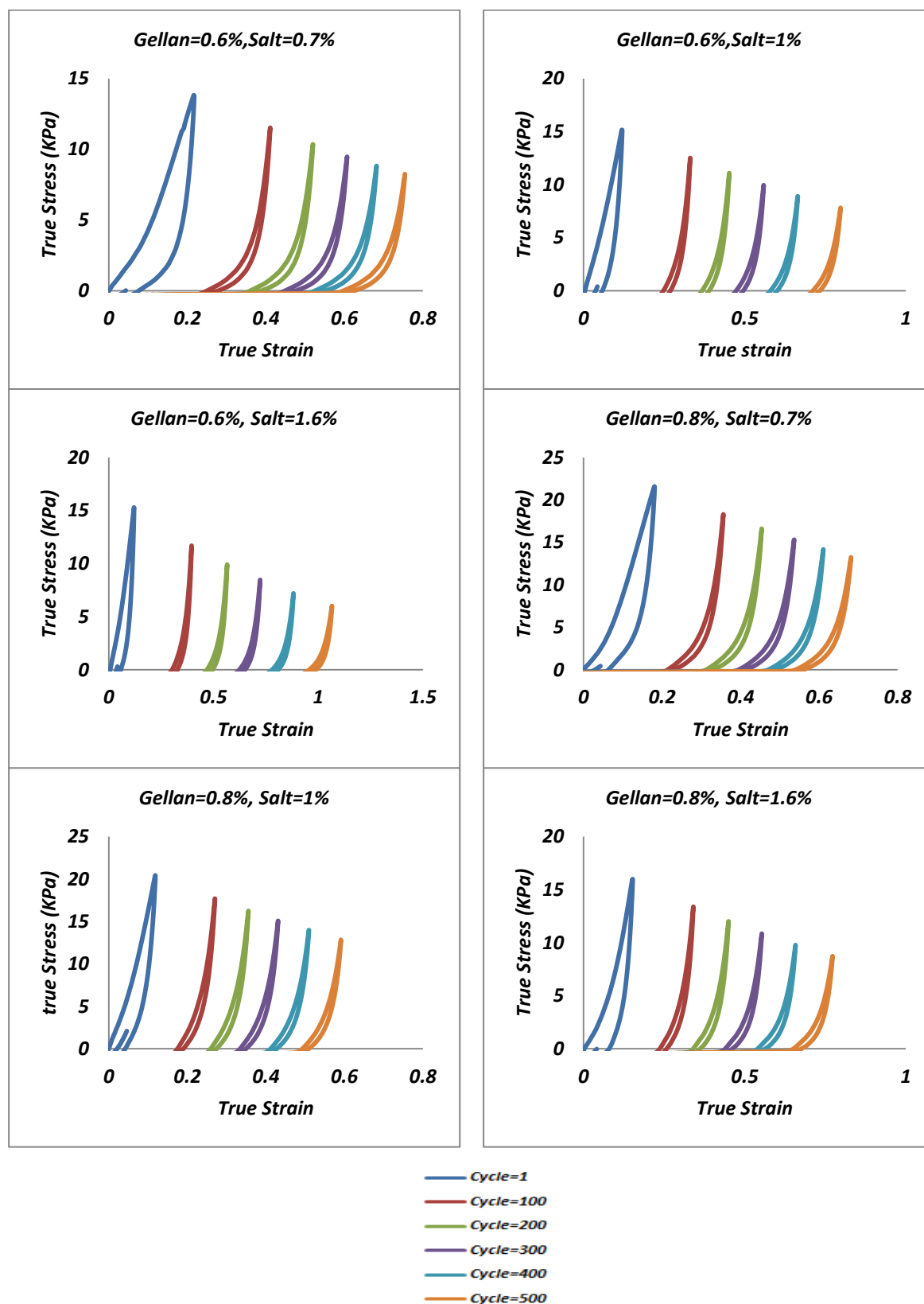


Figure 4.12 True stress versus true strain curve during cyclic compression.

The samples that were selected for this study were subject to five hundred cyclic compressions. After which, each specimen were compressed until failure. As shown in Figure 4.13-Figure 4.16, the mechanical properties of the gellan gels such as true stress and true strain at failure are affected by cyclic compression as a precondition or pre-treatment.

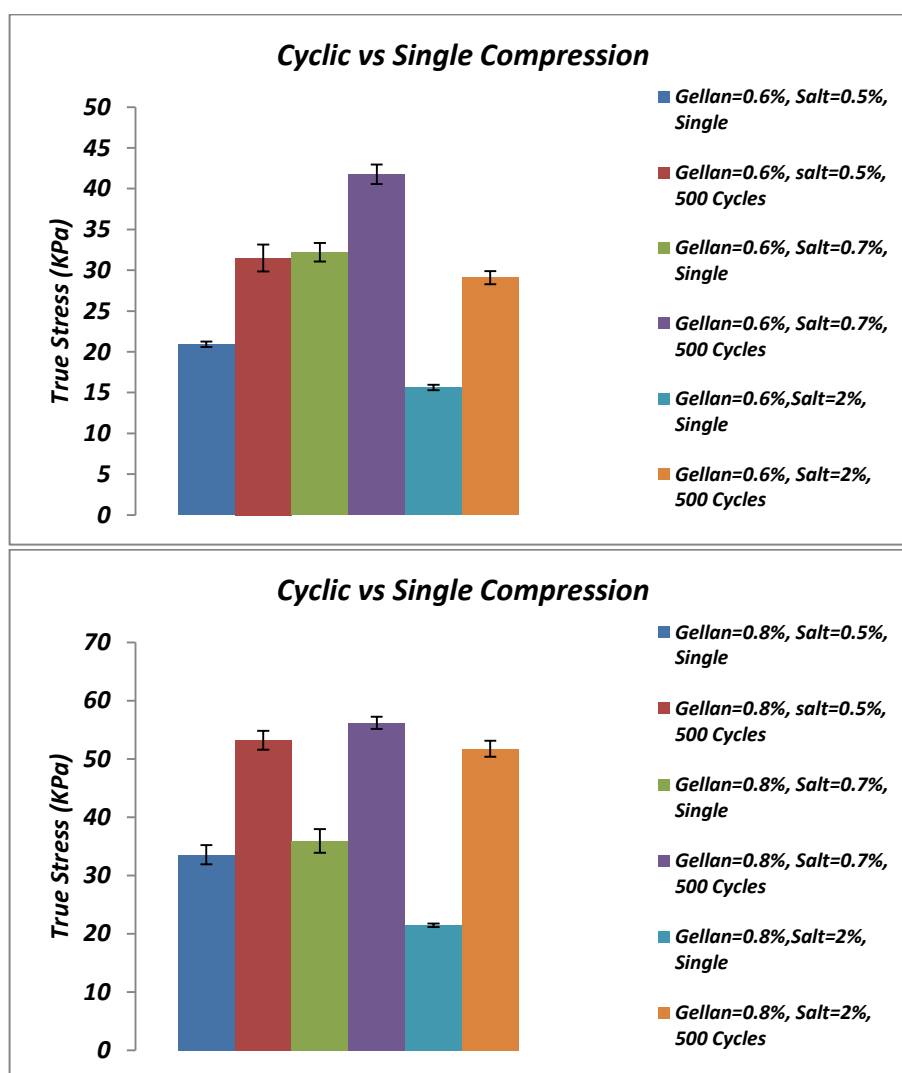


Figure 4.13 Comparison of true stress between single compression and cyclic compression (500 cycles). The error bars show standard deviation from compression of 3 replicates.

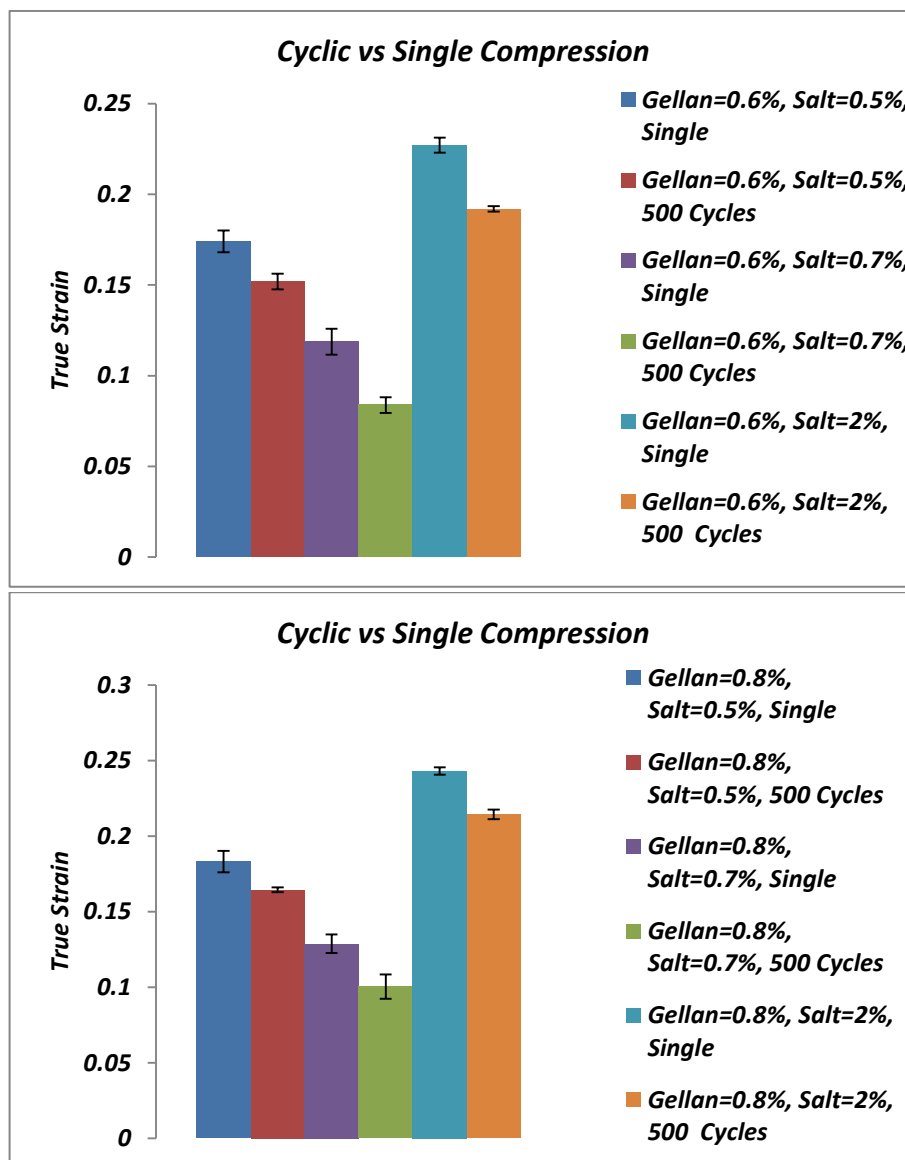


Figure 4.14 Comparison of true strain between single compression and after cyclic compression (500 cycles). The error bars show standard deviation from compression of 3 replicates.

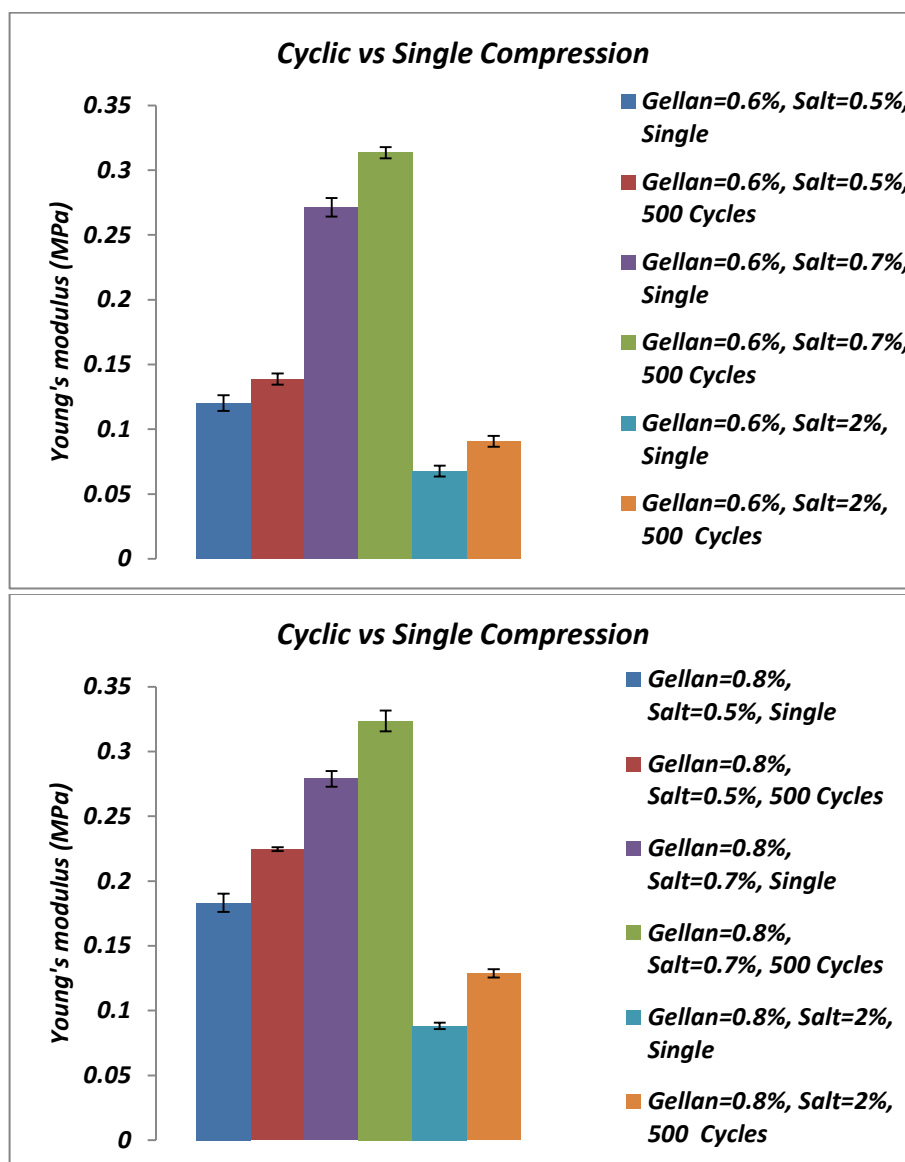


Figure 4.15 Comparison of Young's modulus between single compression and after cyclic compression (500 cycles). The error bars show standard deviation from compression of 3 replicates.

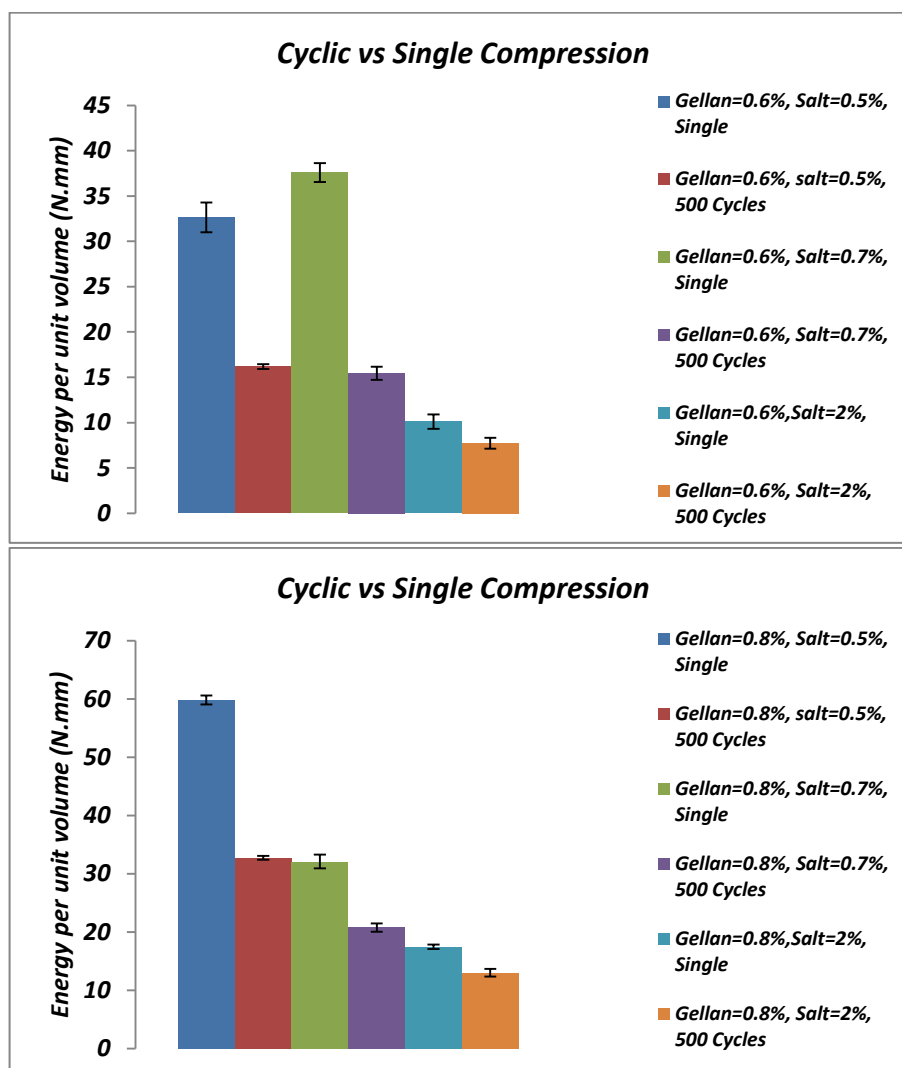


Figure 4.16 Comparison of energy per unit volume between single compression and after cyclic compression (500 cycles). The error bars show standard deviation from compression of 3 replicates.

Figure 4.13 shows the differences between the true stress at the failure point of gellan gels, subjected to 500 cyclic and the specimens with no cyclic compression. The true stress at the failure point of the gellan gels following the cyclic compression was increased as compared to the normal samples. Thus, by cycling, the gellan gels become harder and stronger than normal gels as the strengths of the gels were increased. Water release during cyclic compression caused to change the composition of the specimens, and it can be assumed that water expel following the

cyclic compression leads to new gels with less water content; thus, it seems the new gel contain more gellan gum in its structure; therefore, the strength of the new gel is increased.

Figure 4.14 and Figure 4.15 show the differences between the true stress and Young's modulus at the failure point of gellan gels, which were subjected to five hundred cyclic compressions before uniaxially compression until failure and the specimens with no cyclic compression, respectively. It can be concluded that, by cyclic compression, the true stress at failure was decreased because of reduced water content of the gellan gels; thus, the samples with lower failure true strains represent that the gel exhibits a fracture easily. Also, the Young's modulus was increased after cyclic compression; thus, it can be concluded that the firmness of the samples was increased after cyclic compression, as mentioned above.

Figure 4.16 shows the comparison of energy per unit volume, which is required at failure point between cyclic and single compression. As it was observed energy per unit volume was decreased following the cyclic compression. Therefore, it can be concluded that, by cyclic compression, the strength and firmness were increased, which made the gels harder and stronger, but as energy per unit volume was decreased, the gels become more brittle; therefore, the gels were ruptured more easily.

The energy lost in compression can be characterized by an energy balance equation:

$$E = E_r + E_d + E_f \quad (4.3)$$

where (E) is the total energy lost during compression, (E_r) is the energy that can be used to release the water, (E_d) is the plastic deformation energy that can be used for compression of the gel during cyclic compression and (E_f) is the friction energy due to friction between structural elements of the gels.

Figure 4.17 shows the energy lost during cyclic compression, as it can be observed the majority of energy lost was happened between 1 to 50 cycles, and then after 50 cycles the energy lost remained constant, thus it can be assumed that during cyclic compression (up to 50 cycles) micro cracks in gellan structure were created to help the water to come out through the gel structure then the energy just used to release the water and overcome the friction. Thus decreases in storage Young's modulus are caused by breakdown of the weak secondary bonds like hydrogen bonds of the network structure (Nishinari *et al.*, 1985).

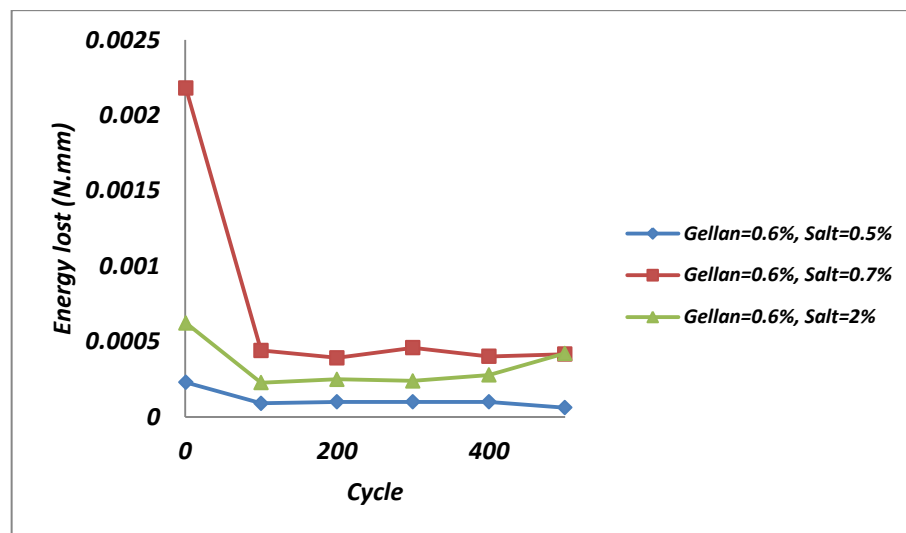


Figure 4.17 Energy lost during cyclic compression.

4.4. Conclusion

In this chapter uniaxial compression was used in order to study the effect of the low acyl gellan gum concentration, salt concentration and cyclic compression on the mechanical properties of the gellan gels. The Instron universal testing machine equipped with a 10 KN load cell was adequate to investigate the mechanical properties of the gellan gels. Mechanical properties parameters such as true stress, true strain, Young's modulus and energy per unit volume were considered.

Firstly, it may be concluded that the cross head speed in selected range does not have any effect on the mechanical properties of the low acyl gellan gum gels.

Secondly, there is not significant different between mechanical properties of immersed and dry gels, therefore, it can be concluded that mechanical properties of the gellan gels are not affected by immersing the gels in the water.

Thirdly, it can be concluded that the low acyl gellan gum concentration play a role on the mechanical properties of the gellan gels. The effects of gellan concentration in all samples with increasing amount of gellan led increased true strain; true stress; Young's modulus and energy per unit volume are observed. The strength of gellan gels increases with gellan concentration at all levels of salt. It happens because of the higher concentration of biopolymer that results in higher cross link densities, in the gel network; therefore, it forms stronger gels.

Fourthly, the salt concentration (as a cross linking agent) play a major role on the mechanical properties of these gels. The gels have a critical salt (cation)

concentration which above this concentration gels are brittle and soft and can have deformation below this concentration. The critical cation concentration of the gels correspond to the state in which all the anionic sites in potential junction zones along the gellan polymers are occupied by cations, so maximum interaction take place within gel network, excessive cations inhibit the formation of cross-links by imposing repulsive forces between polymer molecules, and thereby weaken the gel structure. In all samples can be observed that true strain sharply decreased at 0.7 wt% salt concentration then sudden rise were happened in all samples. The increasing true stress can be observed from 0.5 to 0.7 wt% salt, then sudden drop were happened. As a result the maximum point of true stress can be observed at 0.7 wt% salt. However the salt concentration more than the maximum point which is 0.7 wt% can caused sharply decreased of true stress on all samples. Maximum Young's modules can be seen at 0.7 wt% salt, then sudden drop were happened in all samples. Maximum Young's modules can be seen at 0.7 wt% salts and energy per unit volume is sharply increased and then sudden drop were happened in all samples.

Fifthly, the mechanical properties of the gellan gels can be significantly affected by cyclic compression. As following the cyclic compression plenty amount of water expelled throughout the structure of the gellan gels, therefore, its mechanical properties were significantly altered by cyclic compression. By applying cyclic compression true stress is raised but true strain is dropped, also, Young's modulus is increased but energy per unit volume generally is decreased. Following the cyclic compression more the water comes out from the gels so as a consequence of this effect new composition with different properties are synthesized. As a result of lose

water more dense sample will be made so after cycling compression the gel needs more true stress and less true strain to be ruptured. However Young's modulus is increased and energy per unit volume is decreased at the same point. It can be concluded that the cyclic compression led to more strength and more brittle on the gellan gels.

Chapter 5. Microstructure of the Low Acyl Gellan Gum Gels

5.1. Introduction

The previous chapter studied the effect of the low acyl gellan gum gel concentration, salt concentration and the cyclic compression on the mechanical properties of the low acyl gellan gum gels. As discussed in Chapter 4, the mechanical properties of the gellan gels are dependent to the gellan and the salt (cation) concentration; also, by cyclic compression the mechanical properties of the gellan gels significantly were affected because of expelled water throughout the structure of the gels. It is the objective of this chapter to investigate the microstructure of the low acyl gellan gum gels. This chapter focuses on understanding the effect of the low acyl gellan gum concentration, salt concentration and cyclic compression on the microstructure of the gels by using Cryo Scanning Electron Microscopy (Cryo SEM). This has been achieved by using Cryo Scanning Electron Microscopy to compare the microstructure of specimens with different gellan and salt concentration, also, after cyclic compressions. Several images from low acyl gellan gels with different salt and gellan concentrations were captured, also, the image of the gels after cyclic compression were captured to investigate the effect of cyclic compression on microstructure of the specimens. This chapter will establish, within the microstructure:

- i. The effect of the low acyl gellan gum concentration on microstructure of the gellan gel;

- ii. The effect of the salt (cation) concentration on the microstructure of the gellan gel;
- iii. The effect of the cyclic compression on the microstructure of the gellan gels;

This knowledge and understanding will be important for analysis the flavour release from the low acyl gellan gum gels (Chapter 6).

The deformation of the gels structure during the cyclic compressions results in the release of the serum from the gels. The study of its impact has been majorly carried out on small deformations of the structure from cyclic compressions including the research done on the low acyl gellan gel. Small deformation properties of mixed gels were extensively studied (Aguilera & Stanley n.d.). On the other hand, to develop understanding of this deformation behaviour on a large scale, the microstructure of the gellan gels needs to be studied thoroughly to establish a connection between the microstructure of the gels with the mechanical properties of the gel which gives different observations on separate concentration mix and stages of the cyclic compressions. The serum released and the microstructure of gels after different stages of cyclic compression has not been researched or lengthily tested on experimental basis for gellan gels. The serum released from the gels following the cyclic compression, has various aspects of influence on the gels mechanical properties but its effect on the microstructure of the gels that need clear attention and elaborating studies on the subject are necessary. The serum released after the compressions, results in the change of the geometrical and volume of the gel; it also renders changes to the microstructure of the gels. For this purpose, the serum released from the gels also needs study at an elaborate level to develop more

understanding of the structural changes which it causes to the gel after its release. For this, image analysis of the structure was carried out for observation at all stages.

5.2. Cryo Scanning Electron Microscopy (Cryo SEM)

The gellan gels microstructures consist of distinct zones which are linked with that of the flexible polymer chains. The fibrous model of gellan is, based on the scattering of light and AFM experiments and suggested that the occurrence of non-associated fibrous strands of gellan and lateral aggregation of double-helices which leads to a fibrous microstructure (Ogawa *et al.*, 2005). Considering the gel microstructure is of great significance for process and product development having immense effects on the product attributes. In the present study the samples which contain large volume of water like the gellan gum gels cannot be directly observed by using Scanning Electron Microscopy (SEM) without the removal of water content from their structures. On the other hand, Cryo SEM has the ability to examine the microstructure of hydrated samples. In this work, information about the microstructure of these gels was attained by developing images of the Cryo fractured gels in a Cryo SEM (Fumani *et al.*, 2008).

In this specific technical analysis, the samples are frozen and observed with the help of microscopic studies to develop understanding of their structures at low temperatures. This technique has continually been improved and modified according to the requirements of the tests (Klang *et al.*, 2013). The freeze fracturing can also occur in the specimen under the cryogenic preservation conditions, which may expose the inside microstructure of the sample (Kasas *et al.*, 2003; Ong *et al.*, 2011). The advantage gained from this study is that the hydro content of the system is

maintained within its structure which enables us to study the structures in a better way. Another advantage of this technique is its speed and accuracy in the determination of the observational patterns for making the structural micro-analysis. Furthermore, Cryo SEM has been determined as an excellent platform for the detailed morphologically study of development in its structural form and has many advantages over other methods (Sriamornsak et al. 2008).

5.2.1. Image Analysis

The Cryo SEM micrographs Image analysis with a magnification power of 6000× was carried out using the SKYSCAN software technology. The advantage of using this for scanning the images is that it forms patterns on the basis of contrast between the two phases (pores and solid part) to form the image structure of these stages. Using bars of known lengths, pixel values were converted into distance units. Figure 5.1 shows an example of the scanned image and binarised image of the gels. With the help of that scanned image, the biggest promising rectangular cross-section is taken from the whole image obtained and pore areas are highlighted with the help of this software, SKYSCAN, an illustration of which is shown in Figure 5.2. Using bars of known lengths, pixel values were converted in the form of distance units. The largest possible rectangular cross-section of the samples was cropped. After adjustments to the threshold, area-based pore size distribution, and presentation of the pore-areas as fraction of total area were developed with the help of this software. The porous measurement was done as the fraction of pore-area with respect to the total sample area. The porosity was calculated in the form of a two dimensional pore fraction and it was not showing the absolute porosity of the sample. However, a quantitative comparison of the micrographs obtained from different samples. At higher

concentrations, the gellan chains were closer to each other which enhance the probability of aggregation and junction zones formation. Thus it decreases the time to reach gel point and a more densely linked network structure formation with thicker strands.

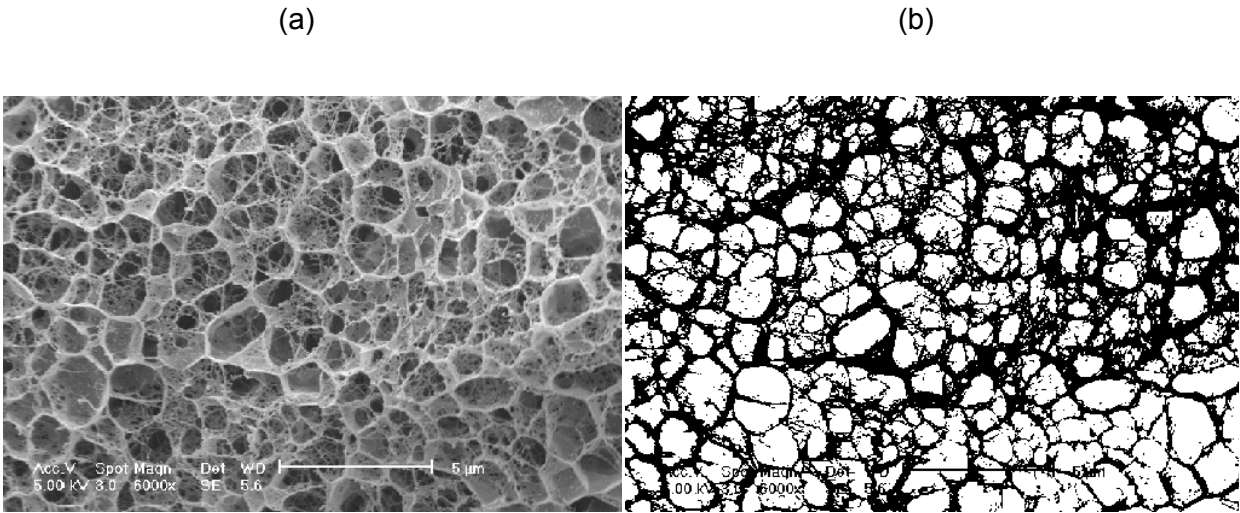


Figure 5.1 (a) Scanned image of gellan gum gel (b) Binarised image of this gel.

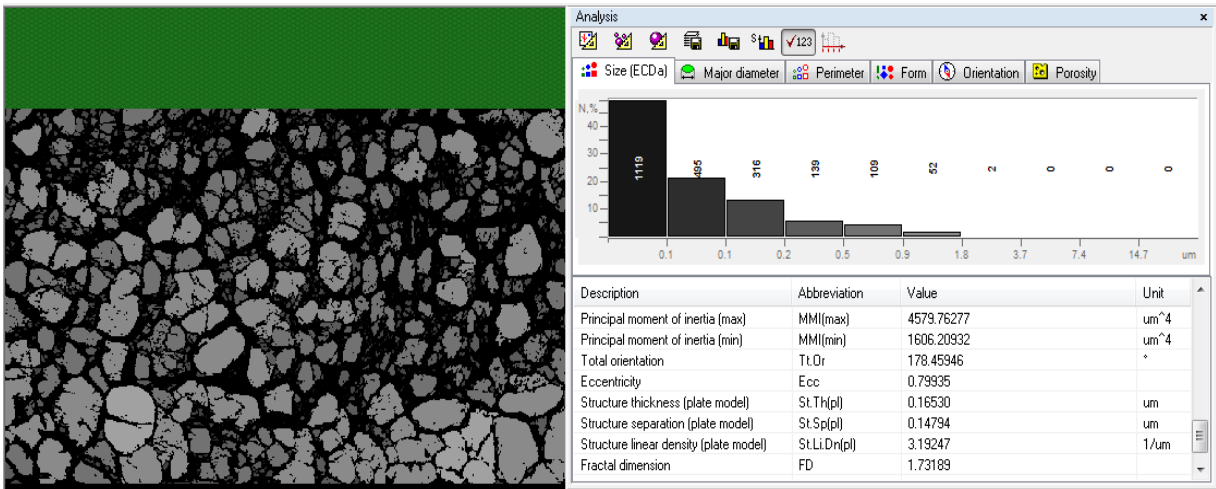


Figure 5.2 One sample of analysed image by SKYSCAN software.

5.3. Cryo SEM Observations

The Philips XL 30 Cryo SEM FEG was used to make the observations for this experimental process with the help of a high resolution device which was able to generate images of the acyl gellan gum gels.

The liquid nitrogen slush was used to freeze the Gellan gels samples under vacuum to approximately -196°C . A thin layer of Gold (10 nm) was then used to cover the samples to make them of conductive nature which helped in carrying out tests on the samples. In the end, an apparatus was set up for the sample to be placed in an environment which was around -140°C to capture the images. The presentation of these results was made on the Cryo SEM micrographs with a magnification level of around 6000times.

Gels microstructure was acquired with the help of this image analysis. By the image analysis, the quantitative data was gathered in the form of area-based pore size distributions and pore-area as fraction of total, while qualitative observations were made about the structure, which determines the character of the gels through this image analysis. The quantified information gained from these observations is given below.

5.3.1. Gels Microstructure

Because of the large water content of the gellan gum gels, Cryo SEM minimized the impacts that alterations to the sample were made for removing the water content of the structure or making alterations to the structure for experimental purposes. The quick freezing process with the help of nitrogen was done to prevent crystallization of

the sample because of the freezing and not to disturb the spatial structure of the whole setup. This prevents distortion of any sorts in the structural pattern of the sample.

The knowledge in the sequence, regarding the microstructure, changes at various stages and pressure cycles of the gellan gels were obtained from the Cryo SEM. One of the significant advantages of using this method for making observations is the preservation of water in the structure, which forms an important component of the gel (Sriamornsak et al. 2008)(Echlin 1992). Therefore, there was no sort of reduction in the volume of the sample structure.

There is a greater chance of artefacts which leads to change in microstructure observation (Hassan *et al.*, 2003). Thus to minimize this situation present utilize Cryo-scanning electron microscopy to characterize the microstructures. This characterization of the microstructure form images helped in understanding the structural pattern layout. The bright areas represented the solid part whereas the dark sides has more liquid component of the overall structure.

5.3.1.1. Effect of Gellan Concentration on the Microstructure of the Gels

Figure 5.4, Figure 5.5 and Figure 5.6 show the microstructure of gellan gels induced by certain salt concentration (sodium chloride as a cross linking agent) with different gellan concentration. Low acyl gellan gum gels formed a homogeneous structure with a great number of pores, which conferred a spongy aspect on the gel network. The pores were homogeneously distributed throughout the structure, and presented a wide range of size forming a structure similar to that observed by (Yamamoto &

Cunha 2007). The different pore sizes of the gellan network could be responsible for the high water holding capacity of the gellan gels. By increasing the gellan gum concentration the shapes of the pores become more uniform, since there was more polysaccharide available to absorb the water (Figure 5.3, Figure 5.4, Figure 5.5 and Figure 5.6) **Figure 5.6** SEM micrographs of low acyl gellan gum gels at 6000× a) gellan gum 1.2% and Salt 1.6% b) gellan gum 1% and salt 1.6% c) gellan 0.8% and salt 1.6% d) gellan 0.6% salt 1.6%.

This led to increase the water holding capacity of the gels when compared to the low gellan concentration gels. Regarding to the previous researches (Mao et al. 2001) it can be assumed that two types of microstructures may exist simultaneously within gellan gels. One microstructure is responsible for the long time stability of the gellan gels, such as water holding capacity, this structure is not affected by cation concentration. A second microstructure, which is depend on cation concentration, is responsible for the gel behaviour when the gels are subjected to external forces.

The gellan gels samples have vary pore size with the gellan gum concentration. As it can be observed in Table 5.1, the porosity can be affected by gellan gum concentration, then porosity decreased with an increasing gellan gum concentration.

Figure 5.3, Figure 5.4, Figure 5.5 and Figure 5.6 showed the Cryo SEM images of the low acyl gellan gum gels with different gellan and salt concentrations. The microstructure of the low acyl gellan gum gels showed a homogeneous structure. As it can be seen in the images, for all specimens, increase of gellan gum concentration led to coarser strands in microstructure. This is visible for all range of salt concentration. Two discrete pore size distributions were observed in the gellan gels.

As it can be observed, the large pores were formed with thick strands of the gel network while the small pores were formed by a thin web structure. The minimum mean pore size was observed at gels with 0.7% salt concentration, corresponding to the critical salt concentration related to the maximum gel strength. Below the critical salt concentration, the mean pore size increased with increasing the salt concentration. Above the critical salt concentration, the mean pore size increased with increasing the salt concentration, and network defect of thick string net were observed.

The spatial structure of the gels was affected by salt concentration. The formation of a regular porous structure in the gels with the addition of salt indicates the cross-link formation. The negative effect of high concentration of salt (cations) on the microstructure of the gellan gels networks is constant with the physical appearance of the gels, where excess amount of salt is responsible for polymer aggregation. This may be due to the saturation of cross-link points, where polymer helices interact, followed by rapid aggregation of polymer chain in close proximity. This may prevent long-range cross-linking of the polymer chains that form a cohesive three dimensional network (Thrimawithana et al. 2011).

For the microstructure analysis the porosity of each sample was studied. Table 5.1 shows the average values obtained for the parameters using the SKYSCAN software for the gel samples. It should be mentioned that to analyze the Cryo SEM images the pores with equivalent circle diameter less than $0.3\ \mu$ were filtered and just the pores with above $0.3\ \mu$ equivalent circle diameter have been considered. It can be observed from the table that total porosity was calculated for each image as a

representation of the percentage total pore content within the sample. It can be noted that samples with different gellan concentrations, the sample with 0.6% gellan concentration has the highest porosity value and that initially porosity value decrease with increasing gellan concentration.

Table 5.1 Values of the geometric parameters of the low acyl gellan gum gels containing different gellan concentrations.

Sample		Mean pore size (μ)	Largest pore (μ)	Total Porosity %
Salt=0.5%	Gellan=0.6%	0.97 \pm 0.011	3.19	37.93
	Gellan=0.8%	0.77 \pm 0.041	1.95	36.56
	Gellan=1%	0.72 \pm 0.017	2.59	34.99
	Gellan=1.2%	0.67 \pm 0.008	2.11	32.03
Salt=0.7%	Gellan=0.6%	0.51 \pm 0.029	1.54	51.62
	Gellan=0.8%	0.63 \pm 0.019	3.02	48.92
	Gellan=1%	0.54 \pm 0.008	1.37	48.81
	Gellan=1.2%	0.52 \pm 0.007	1.21	45.51
Salt=1%	Gellan=0.6%	0.72 \pm 0.034	2.79	49.06
	Gellan=0.8%	0.65 \pm 0.014	2.94	44.05
	Gellan=1%	0.62 \pm 0.013	2.23	43.93
	Gellan=1.2%	0.64 \pm 0.011	1.82	42.95
Salt=1.6%	Gellan=0.6%	0.70 \pm 0.022	2.07	47.28
	Gellan=0.8%	0.87 \pm 0.006	3.28	46.89
	Gellan=1%	0.64 \pm 0.016	1.74	42.99
	Gellan=1.2%	0.57 \pm 0.005	1.29	42.18

As shown in chapter 4 the results indicate that with increasing gellan gum concentration, both of the Young's modulus and true stress at failure point are increased. It is known that compressive strength and Young's modulus increase with decreasing porosity (Jang & Matsubara 2005). In addition, previous studies have indicated that the Young's modulus of the porous sample significantly increase as the amount of material in the network also increases. Therefore, the mechanical properties of gellan gum gels are primarily determined by the concentration of gellan gum (Gohel et al., 2009; Chang *et al.*, 2012).

When the microstructure of the gellan gels with different gellan concentration was taken into consideration, pores were mostly in spherical like shapes. Figure 5.3, Figure 5.4, Figure 5.5 and Figure 5.6 show the microstructure of the gels in different gellan concentrations at 6000 \times magnification. From the general view, it can be said that the pores of the gels with lower gellan concentration were found to be bigger than those with higher gellan concentration.

It was seen from Figure 5.3, Figure 5.4, Figure 5.5 and Figure 5.6 that the microstructure of the gellan gels with lower amount of gellan concentration seemed to have more porosity than that of higher ones. The pore area fractions of the gels with different gellan concentrations can be observed in the above mentioned figures. It can be seen that the highest pore area fractions were obtained for gels with lower gellan concentration, on the other hand, by increasing the gellan concentration the size of the pores were decreased. It can be assumed that an increase in gellan concentration led to increase the thickness of the solid phase in the gels, thus, the porosity of the gel is decreased then the gels becomes more strength. As it can be observed that the gellan concentration plays a positive role on improving the gels microstructure that makes the gel stronger in nature. The strength of gel increases when salt is gradually added however when the concentrations of salt surpass a critical level the strength finally decreases. Similar findings were reported for Na⁺ gellan added with NaCl while comparing the salt-dependence of (σ_b) with storage modulus (G) (Morris et al., 1999).

On the other hand the gellan gels showed a sponge structure, while an increase of the gellan concentration led to a more compact and interconnected structure. These

results are consistent with those obtained for the mechanical properties in chapter 4, because an increase in gellan concentration led to an increase in true stress at failure point, characteristic of a denser network.

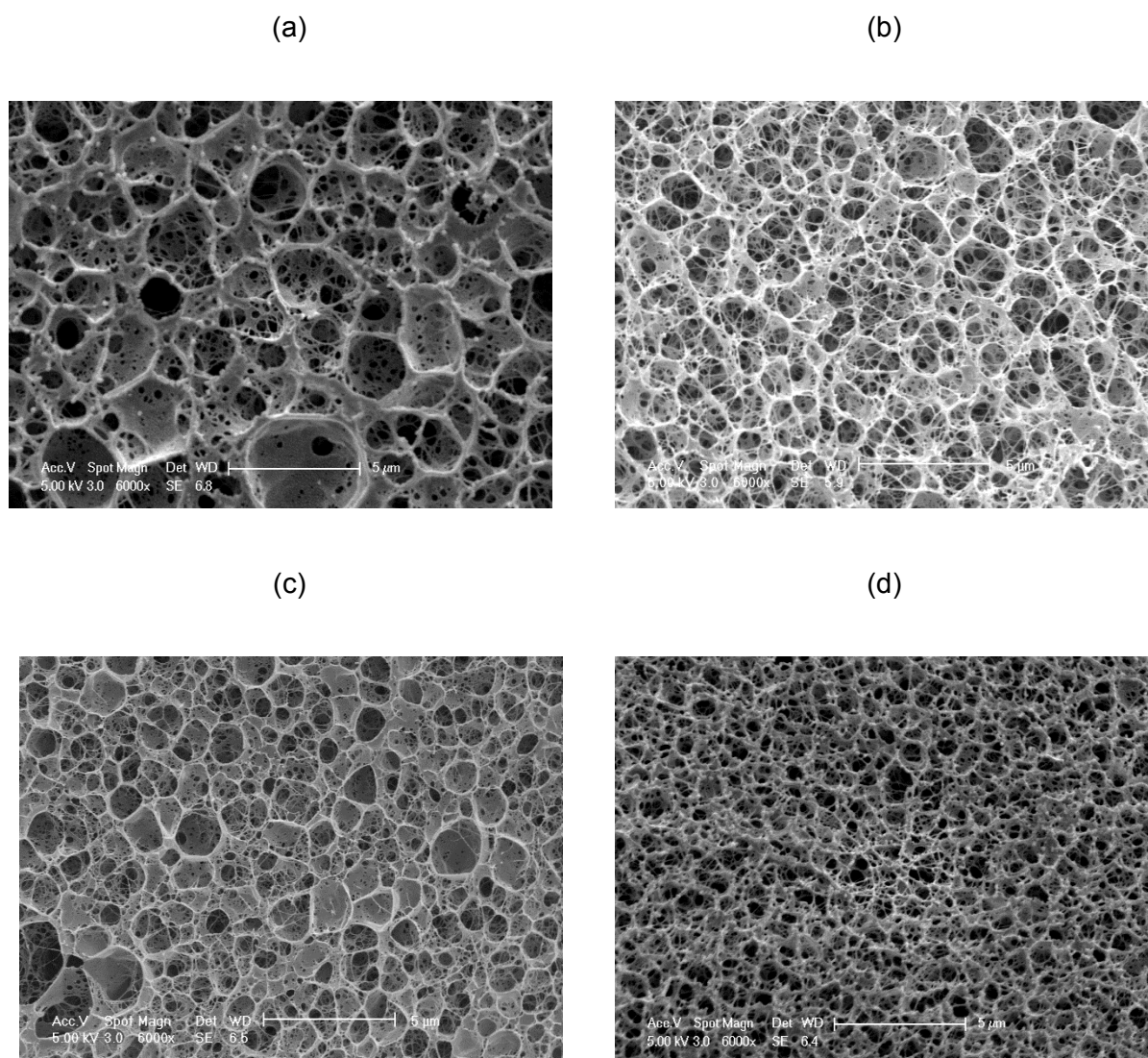


Figure 5.3 SEM micrographs of low acyl gellan gum gels at 6000× a) gellan gum 1.2% and Salt 0.5% b) gellan gum 1% and salt 0.5% c) gellan 0.8% and salt 0.5% d) gellan 0.6% salt 0.5%.

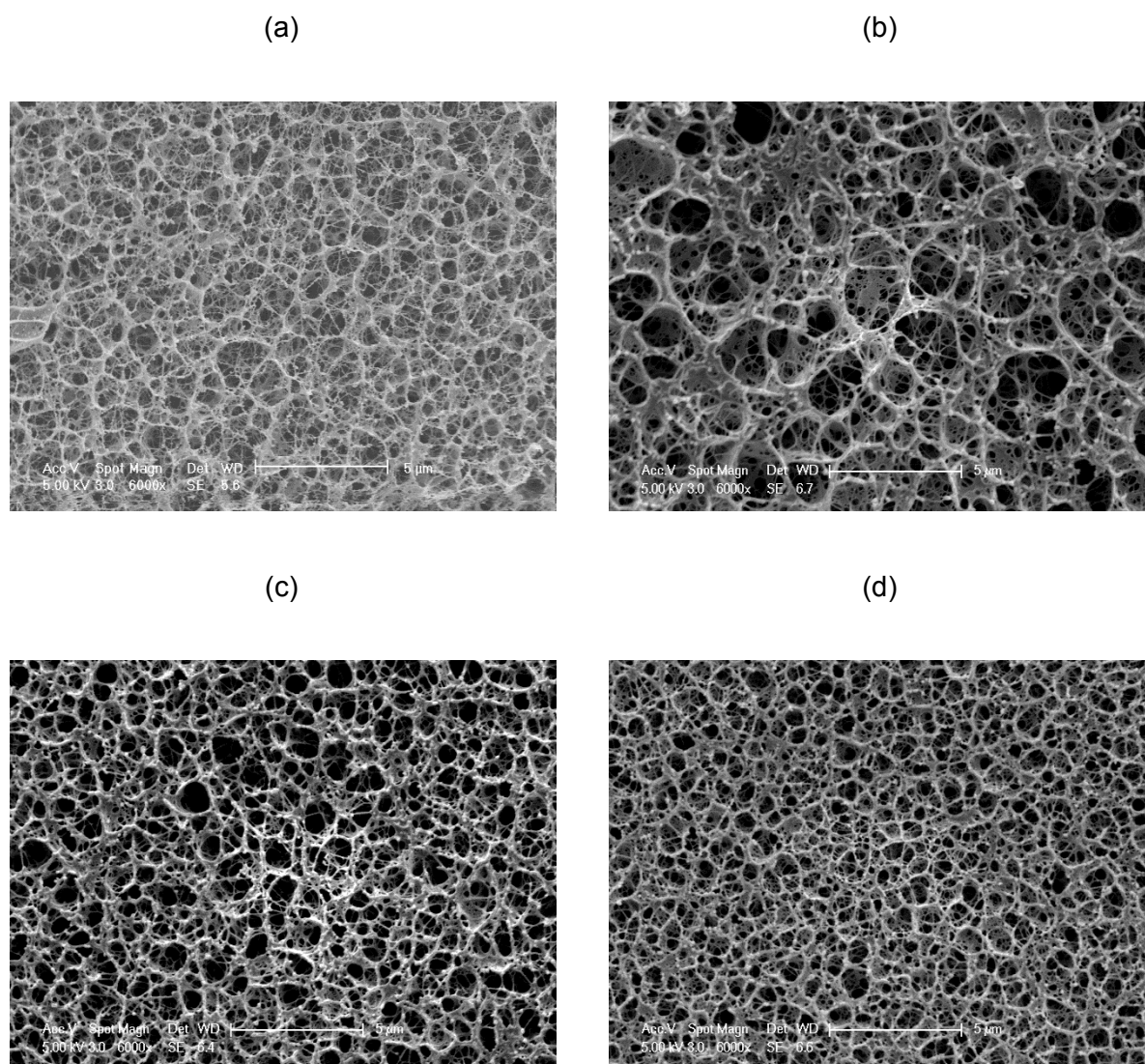


Figure 5.4 SEM micrographs of low acyl gellan gum gels at 6000× a) gellan gum 1.2% and Salt 0.7% b) gellan gum 1% and salt 0.7% c) gellan 0.8% and salt 0.7% d) gellan 0.6% salt 0.7%.

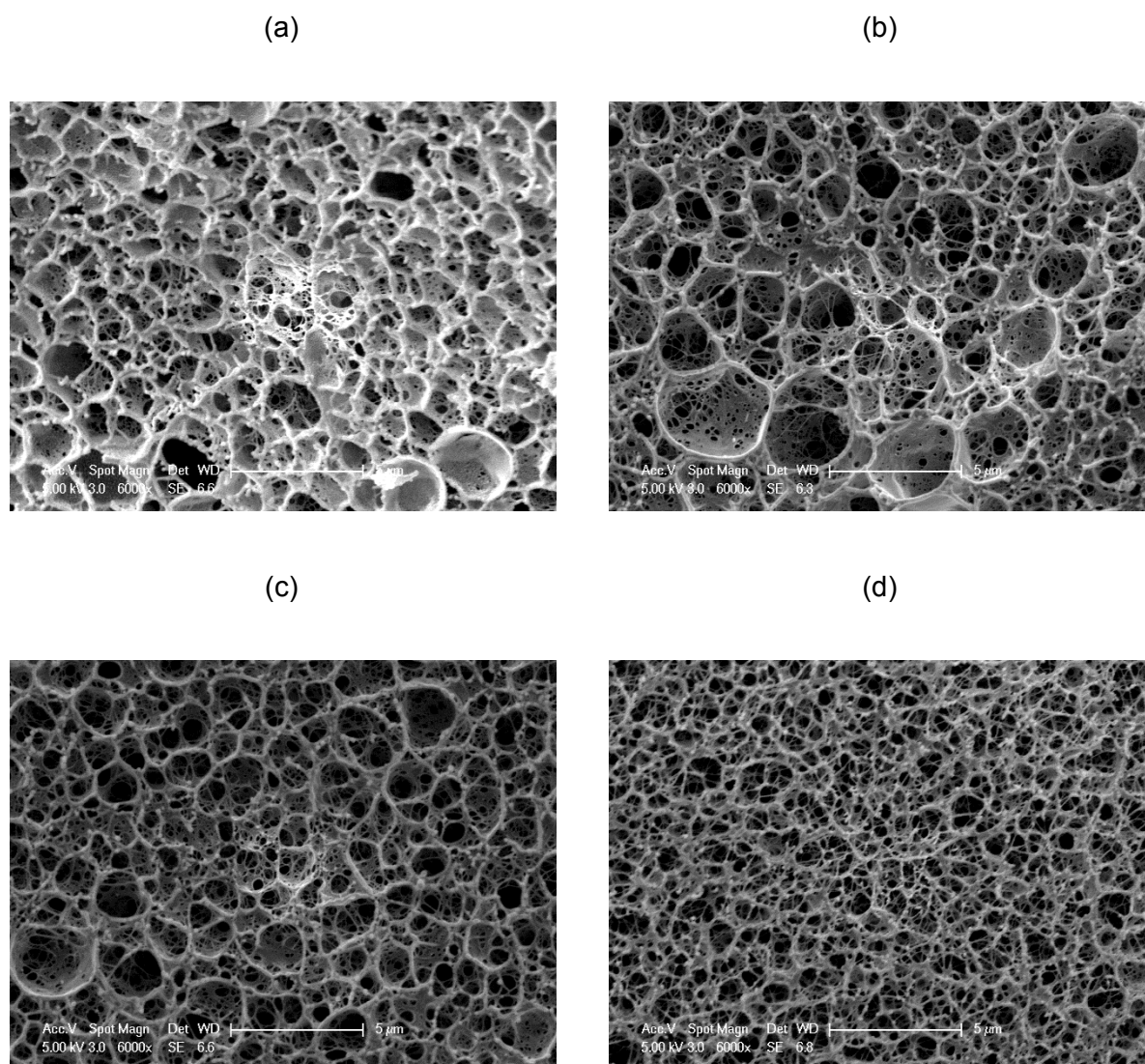


Figure 5.5 SEM micrographs of low acyl gellan gum gels at 6000 \times a) gellan gum 1.2% and Salt 1% b) gellan gum 1% and salt 1% c) gellan 0.8% and salt 1% d) gellan 0.6% salt 1%.

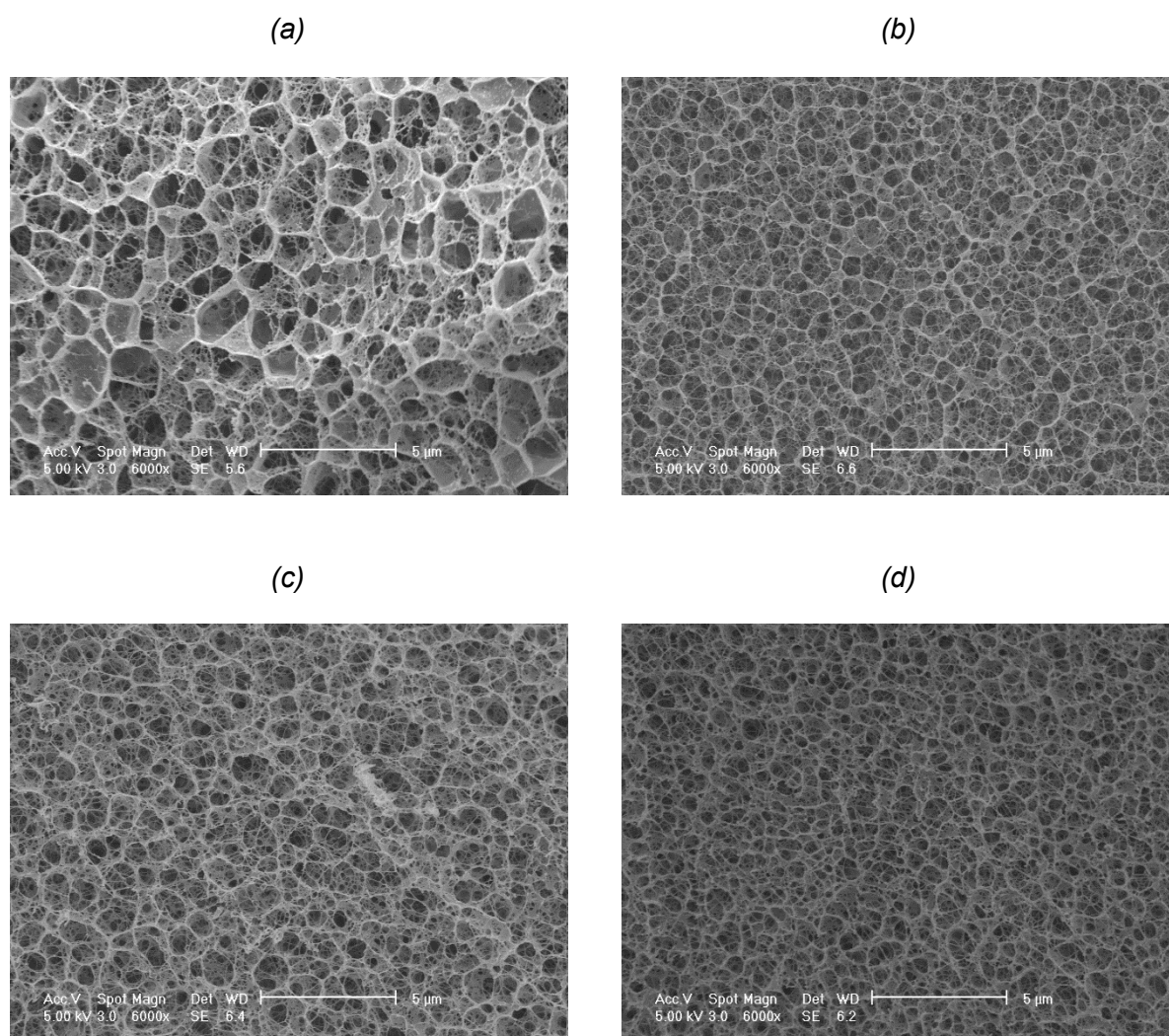


Figure 5.6 SEM micrographs of low acyl gellan gum gels at 6000× a) gellan gum 1.2% and Salt 1.6% b) gellan gum 1% and salt 1.6% c) gellan 0.8% and salt 1.6% d) gellan 0.6% salt 1.6%.

5.3.1.2. Effect of Salt Concentration on the Microstructure of the Gels

To investigate the effect of the salt concentration on the microstructure of the low acyl gellan gels the Cryo SEM was carried out to capture the images of the samples. The microstructure of the gellan gels containing different salt concentrations was observed. Quantitative information in terms of total porosity, mean pore size, and the largest pore size were obtained by the help of image analysis.

Captured figures showed examples of the set of the flat cross sections that were obtained for each sample after binarization of the images using the reconstruction software SKYSCAN; the void spaces in the images, represented by the white areas, are clearly visible. From these images the two-dimensional reconstructions were obtained from which the geometrical parameters were calculated using the SKYSCAN software.

For the micro structural analysis, the pore size structure of each sample was studied. Table 5.2, shows the average values were obtained for the tomographical parameters using the SKYSCAN software. The total porosity value, mean pore size, and the largest pore size were carried out from the gellan gels with different salt concentrations. As it can be observed that the salt concentration was affected the percent number of pores for all pore size ranges significantly (Lee & Larson, 2008). It can be noted that for the gellan gels, there is two district trends, the gels with lower salt concentration below the critical point has low porosity value and that initially porosity value increases with increase in salt content up-to critical point. Whereas for the gels above the critical point has lower porosity value and that initially porosity value decreases with increase in salt concentration. The results of this table

confirmed that above critical point an increase in salt content causes also a decrease in the porosity value for all the gellan gels, therefore the gellan gels become less brittle.

Table 5.2 Values of the geometric parameters of the low acyl gellan gum gels containing different salt concentrations

Sample		Mean pore size (μ)	Largest pore (μ)	Total Porosity %
Gellan=0.6%	Salt=0.5%	0.97 \pm 0.011	3.19	37.93
	Salt=0.7%	0.51 \pm 0.029	1.54	51.62
	Salt=1%	0.72 \pm 0.034	2.79	49.06
	Salt=1.6%	0.70 \pm 0.022	2.07	47.28
Gellan=0.8%	Salt=0.5%	0.77 \pm 0.041	1.95	36.56
	Salt=0.7%	0.63 \pm 0.019	3.02	48.92
	Salt=1%	0.65 \pm 0.014	2.94	44.05
	Salt=1.6%	0.47 \pm 0.006	0.99	46.89
Gellan=1%	Salt=0.5%	0.72 \pm 0.017	2.59	34.99
	Salt=0.7%	0.54 \pm 0.008	1.37	48.81
	Salt=1%	0.62 \pm 0.013	2.23	43.93
	Salt=1.6%	0.64 \pm 0.016	1.74	42.99
Gellan=1.2%	Salt=0.5%	0.67 \pm 0.008	2.11	32.03
	Salt=0.7%	0.52 \pm 0.007	1.21	45.51
	Salt=1%	0.64 \pm 0.011	1.82	42.95
	Salt=1.6%	0.50 \pm 0.005	1.29	42.18

Figure 5.7 shows the sample graph of scanned image of gellan gels containing different salt concentrations. The binarised image that was obtained by the SKYSCAN software can be seen in Figure 5.1. Binarised images were used for quantitative analysis of pores. Porosity value of the gellan gels containing different salt concentrations were shown in Figure 5.7, Figure 5.8, Figure 5.9 and Figure 5.10. Figure 5.7 SEM micrographs of low acyl gellan gum gels at 6000 \times a) gellan gum 0.6% and Salt 0.5% b) gellan gum 0.6% and salt 0.7% c) gellan 0.6% and salt 1% d) gellan 0.6% salt 1.6%.

As it can be observed the salt concentration play a main role on the microstructure of the gellan gels and the porosity value was affected by the salt concentration. Higher porosity values mean that the gels were more porous. Regarding to the results which were obtained in Chapter 4, two discrete effects could be observed by increasing the salt concentration. The first one is below the critical salt concentration and second one above that. Below the critical salt concentration, by increasing the salt concentration up to the critical point the gels showed higher porosity value, however, above the critical point, an increase in salt concentration led to decrease the porosity values. Above the critical point, the gels containing higher salt concentrations were shown to have lower porosity value. These results can be related to the microstructure and mechanical properties of the low acyl gellan gum gels. In chapter 4, above the critical point true stress of the gellan gels containing higher level of the salt, at failure point were found to be lower than the gels which containing higher salt concentration. Higher salt concentration led to increase the repulsive force between the gellan strands then, might help absorb more water into the gels and causes lower porosity value.

When the salt concentrations were compared, above the critical concentration the gels with higher salt concentration showed lower porosity value. The reason for this different salt concentration can be the difference in gelling mechanism. On the other word, above the critical point, an increase in the salt concentration led to decrease the porosity value, then, the gel becomes less brittle. On the other hand, below the critical point by increasing salt concentration up to critical point the porosity value was increased, therefore, the gel become more brittle. These results are consistent with those obtained for the mechanical properties in chapter 4, because the increase

in salt concentration above the critical salt concentration led to a decrease in true stress at failure, on contrary, an increase in salt concentration below the critical salt concentration causes higher true stress at failure point.

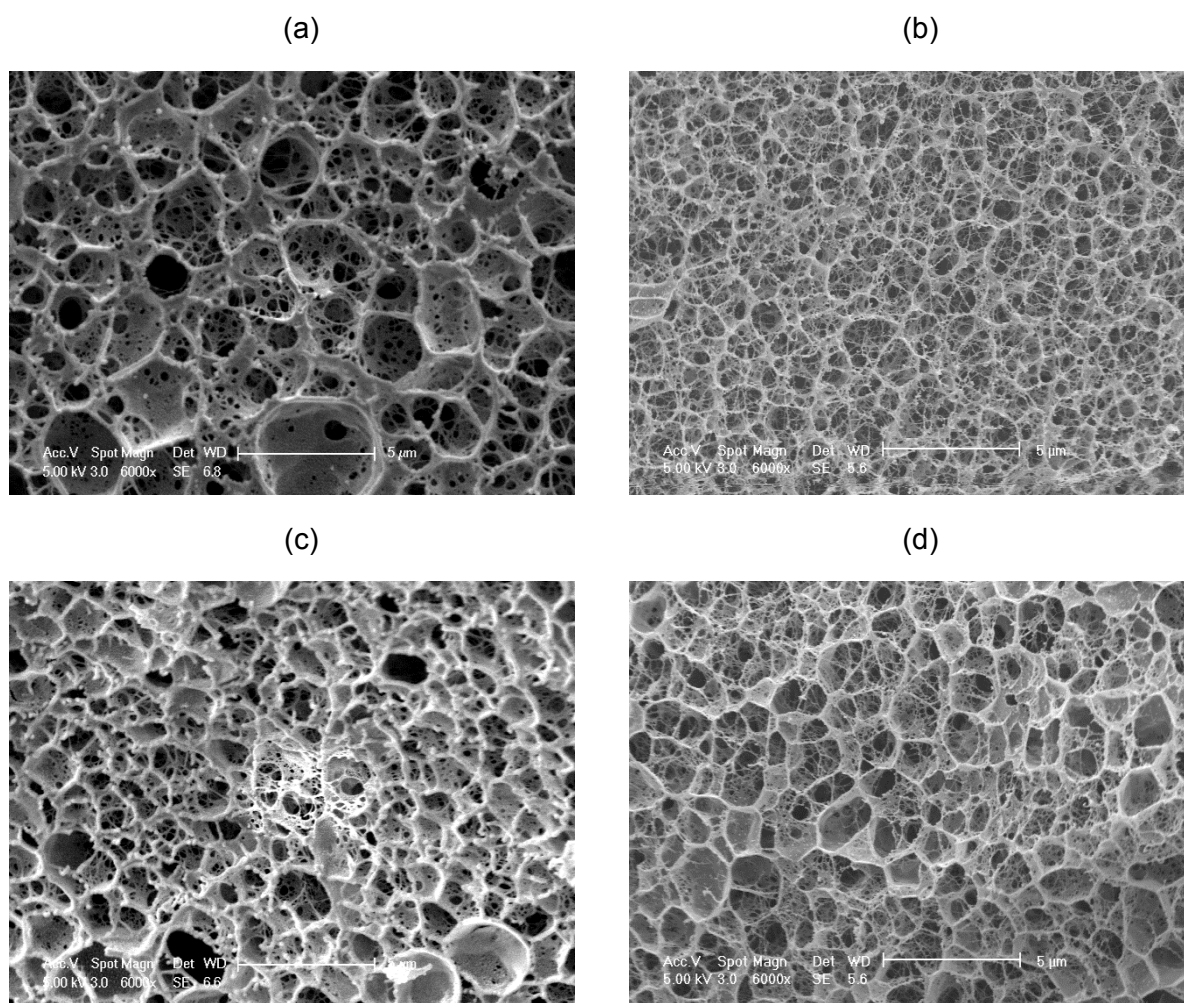


Figure 5.7 SEM micrographs of low acyl gellan gum gels at 6000× a) gellan gum 0.6% and Salt 0.5% b) gellan gum 0.6% and salt 0.7% c) gellan 0.6% and salt 1% d) gellan 0.6% salt 1.6%.

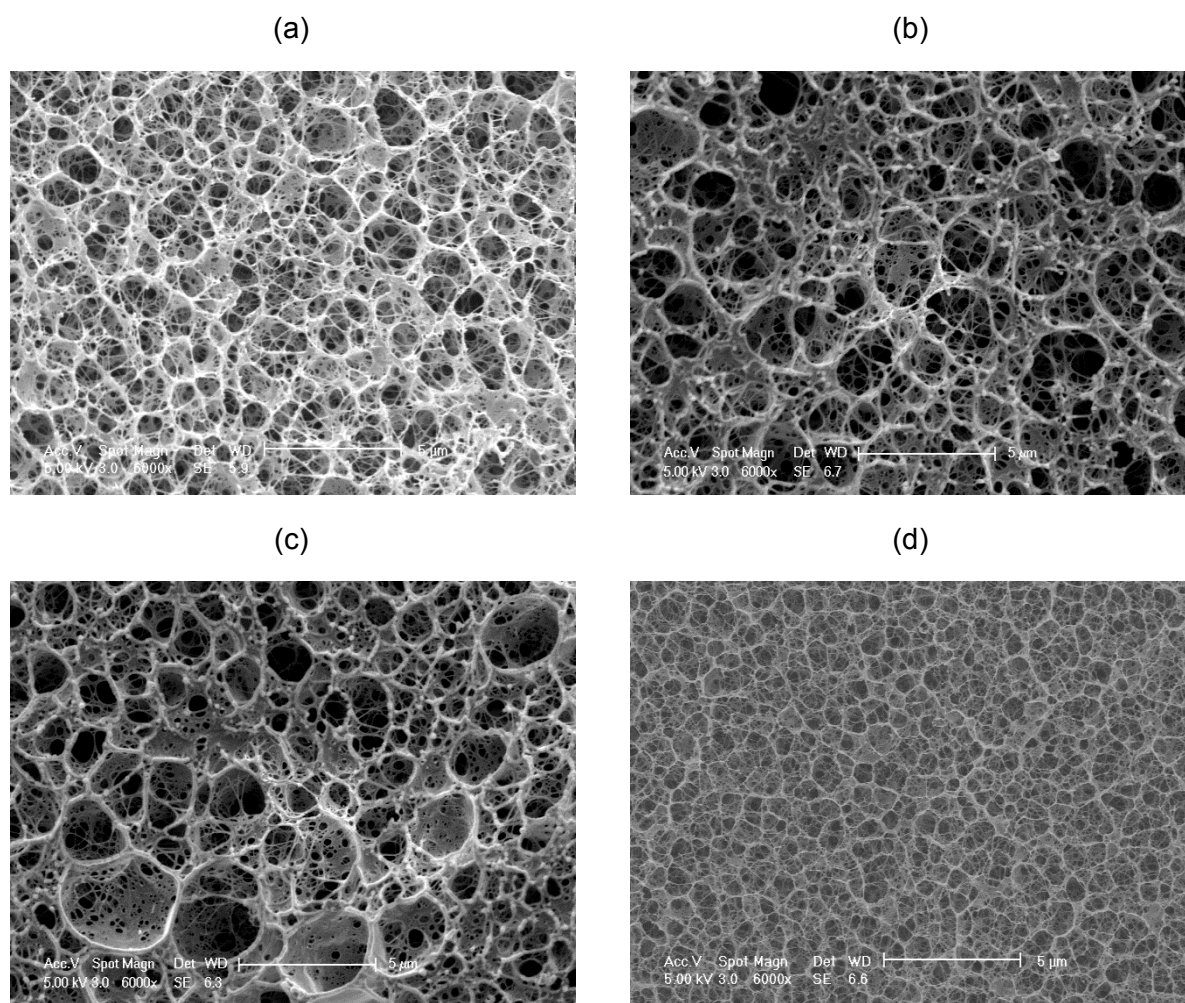


Figure 5.8 SEM micrographs of low acyl gellan gum gels at 6000× a) gellan gum 0.8% and Salt 0.5% b) gellan gum 0.8% and salt 0.7% c) gellan 0.8% and salt 1% d) gellan 0.8% salt 1.6%.

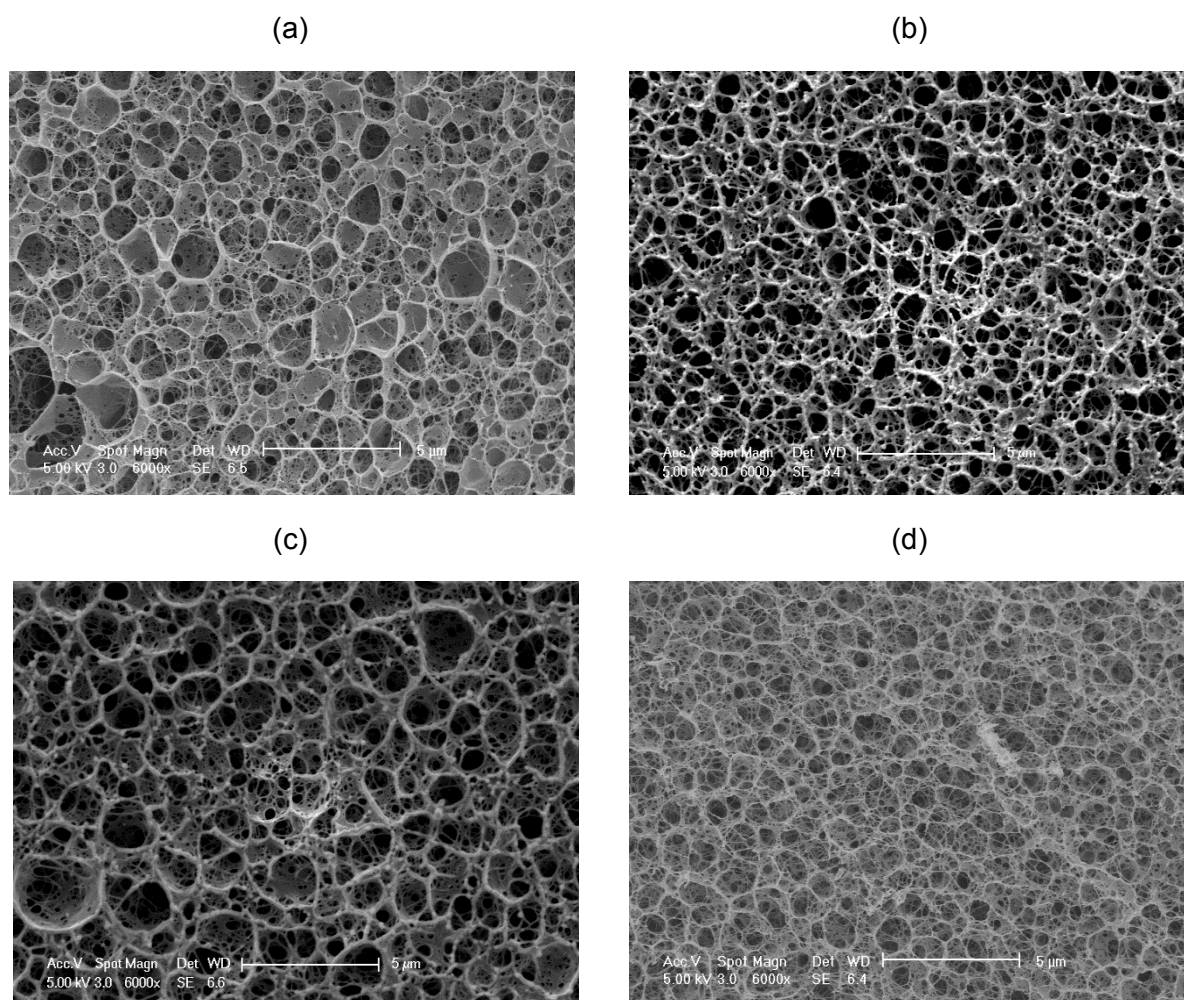


Figure 5.9 SEM micrographs of low acyl gellan gum gels at 6000× a) gellan gum 1% and Salt 0.5% b) gellan gum 1% and salt 0.7% c) gellan 1% and salt 1% d) gellan 1% salt 1.6%.

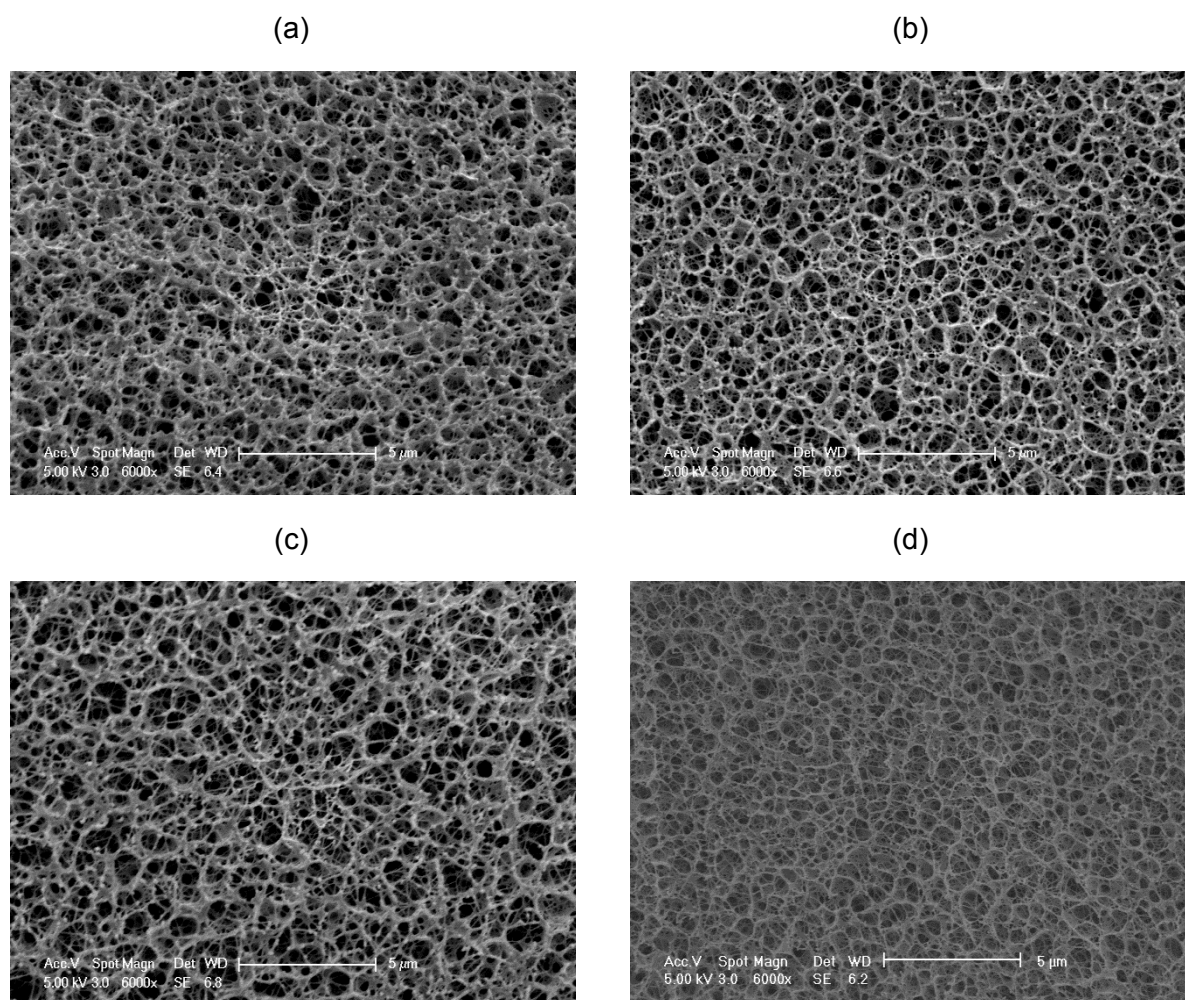


Figure 5.10 SEM micrographs of low acyl gellan gum gels at 6000× a) gellan gum 1.2% and Salt 0.5% b) gellan gum 1.2% and salt 0.7% c) gellan 1.2% and salt 1. % d) gellan 1.2% salt 1.6%.

5.3.1.3. Effect of the Cyclic Compression on the Microstructure of the Gels

Microstructures of the gels after cyclic compression were investigated with SKYSCAN image analysis software. Bright areas in the Cryo SEM images represent the solid phase (low acyl gellan gum) and dark areas represent the serum phase (water). The area of the solid phase was expressed as fraction of the total image area.

Figure 5.11, Figure 5.12 and Figure 5.13 show the images of microstructure of the gellan gels after cyclic compression test. The gels did not fracture during cyclic compression. Instead, they deformed releasing a plenty amount of water. The final gel remained macroscopically intact after the cyclic compression. There were no cracks visible on the outside of the gels as it was shown in Figure 4.8 in chapter 4. The microstructure of the deformed gel is different from the initial gel. It can be observed that the gels showed a great change in microstructure after cyclic compression. When the cyclic compression is subjected on the gels, the microstructure of the gel may carry out restructuration with expelling water throughout the gel. The gellan matrix is deformed and pores in the matrix have become smaller compared with the initial gel. This is related to the water release during cyclic compression. As the water is released, the gellan network collapsed. It can be assumed that the gellan network is disrupted at several places, however, no visual fracture was observed, micro cracks were formed in the gels prior to the fracture point. Therefore, the network and microstructure become more compact with a smaller mean diameter of the pores. After cyclic compression the size of the pores as well as their connectivity is decreased. The decrease in pore size led to lower

porosity, therefore, structures of porosity is decreased at high water release after cyclic compression which led to a levelling off of the water release.

The microstructure of the gel allowed the water to be released during the cyclic compression. This caused the volume fraction of the serum phase decreased and the sample become indeed denser. The microstructure of the gels after cyclic compression was high when compared to the initial gels.

Table 5.3 shows that the mean pore size is changed significantly with number of cycles. This result confirms that the mean pore size, porosity value and strand thickness can be affected by the cyclic compression. This is because when the gel subjected to the cyclic compression, the water content of the gel can be expelled throughout the gel structure, the gel network can be restructured by compacting the strands, then the strands become closer together, producing a tighter network with pores progressively smaller. It can affect the mechanical properties such as hardness, strength and brittleness.

Table 5.3 Values of the geometric parameters of the low acyl gellan gum gels after number of cyclic compression.

<i>Sample</i>	<i>Cycles</i>	<i>Mean pore size (μ)</i>	<i>Largest pore (μ)</i>	<i>Total Porosity %</i>
<i>Gellan=0.8% , Salt=0.7%</i>	<i>50</i>	0.40 \pm 0.005	1.3	50.77
	<i>100</i>	0.36 \pm 0.003	0.89	54.22
	<i>300</i>	0.34 \pm 0.013	0.51	59.22
	<i>500</i>	0.61 \pm 0.014	1.19	66.11
<i>Gellan=0.8%, Salt=1%</i>	<i>50</i>	0.38 \pm 0.018	0.84	49.61
	<i>100</i>	0.74 \pm 0.031	1.43	53.97
	<i>300</i>	0.38 \pm 0.018	0.87	57.11
	<i>500</i>	0.43 \pm 0.032	1.14	64.15
<i>Gellan=0.8%, Salt=1.6%</i>	<i>50</i>	0.38 \pm 0.003	0.78	49.14
	<i>100</i>	0.43 \pm 0.004	0.96	53.18
	<i>300</i>	0.37 \pm 0.002	0.65	56.49
	<i>500</i>	0.35 \pm 0.005	0.55	63.55

The information about the microstructure of the low acyl gellan gels after cyclic compression was gained from Cryo SEM image and image analysis. It can be concluded that significant variation in spatial structure of the samples was observed before and after cyclic compression.

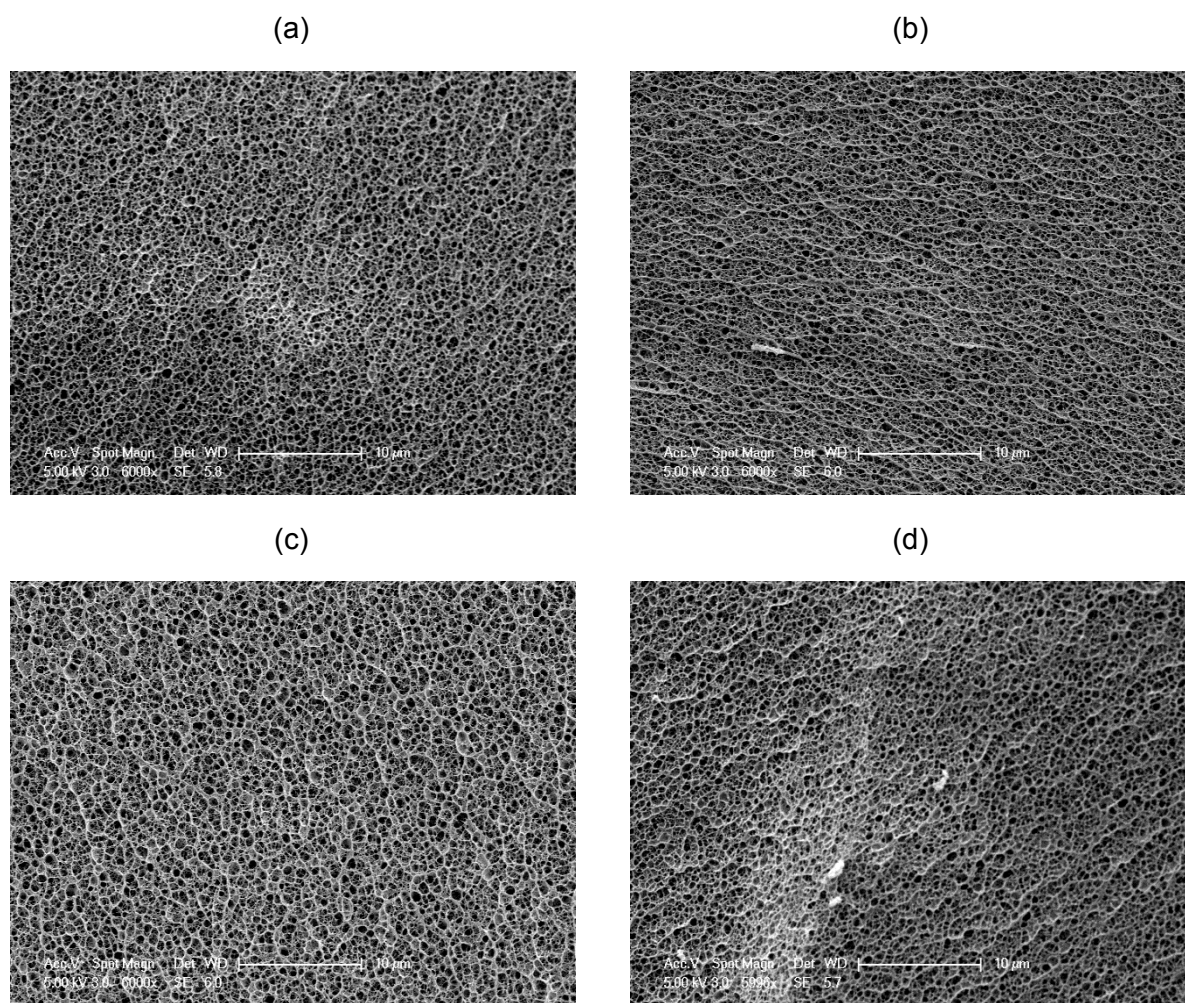


Figure 5.11 SEM micrographs of low acyl gellan gum gels containing 0.8% gellan, 0.7% salt at 6000 \times a) after 50 cyclic compression b) after 100 cyclic compression c) after 300 cyclic compression d) after 500 cyclic compression.

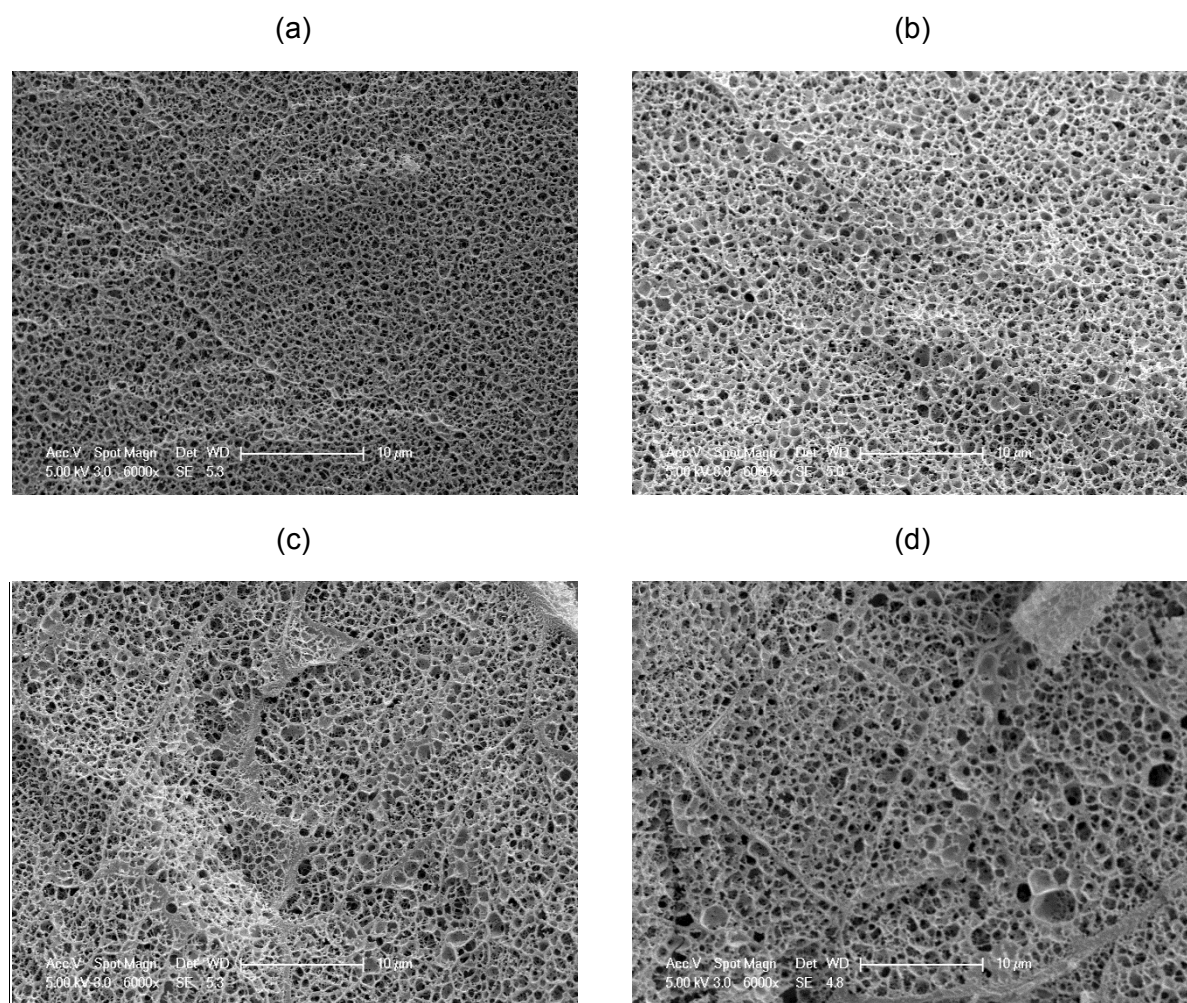


Figure 5.12 SEM micrographs of low acyl gellan gum gels containing 0.8% gellan, 1% salt at 6000× a) after 50 cyclic compression b) after 100 cyclic compression c) after 300 cyclic compression d) after 500 cyclic compression.

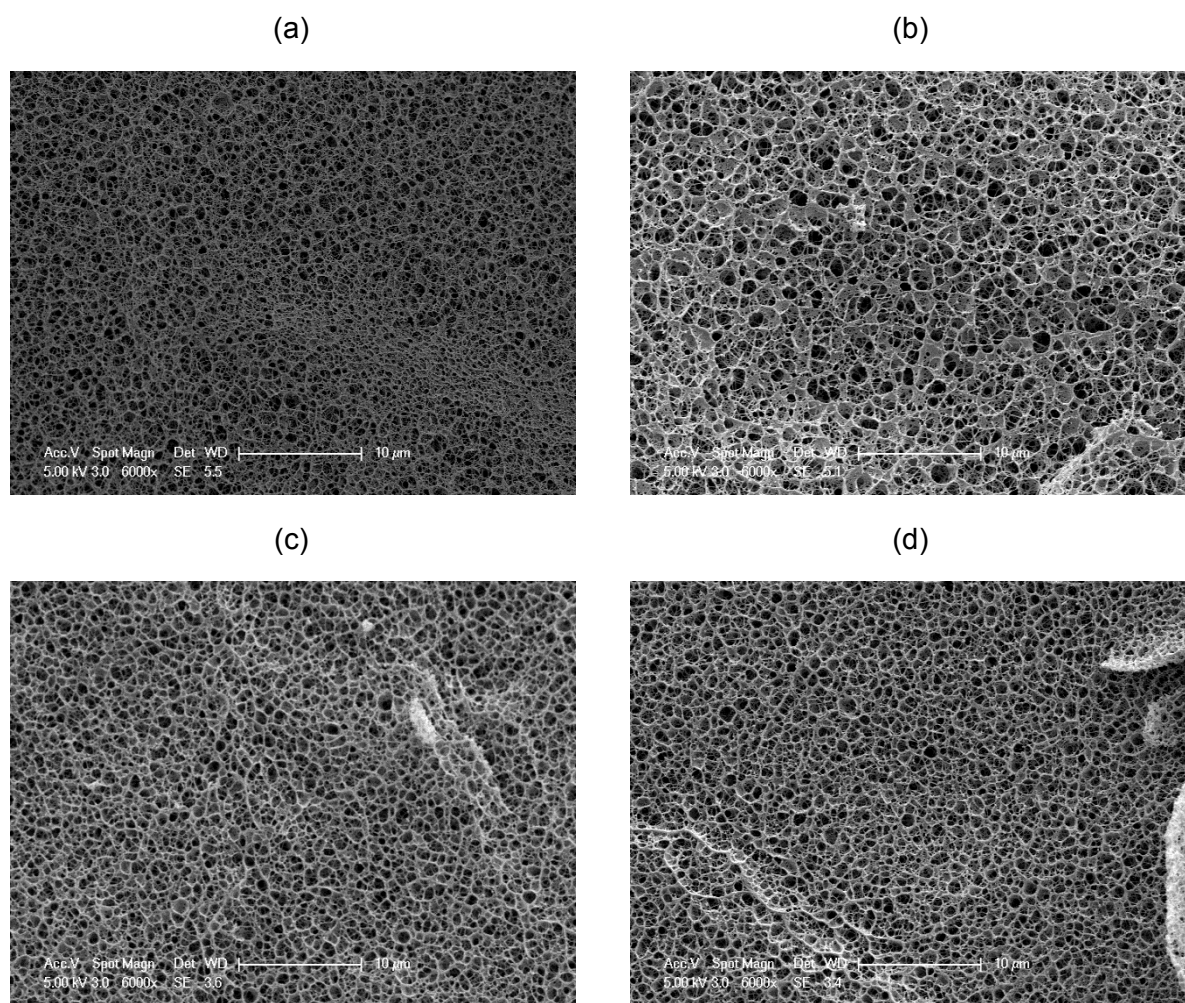


Figure 5.13 SEM micrographs of low acyl gellan gum gels containing 0.8% gellan, 1.6% salt at 6000× a) after 50 cyclic compression b) after 100 cyclic compression c) after 300 cyclic compression d) after 500 cyclic compression.

5.4. Conclusion

In this chapter image analysis was used in order to study the effect of the low acyl gellan gum concentration, salt concentration and cyclic compression on the microstructure of the gellan gels. The SKYSCAN software was adequate to carry out microstructural analysis of the gellan gels. Microstructural parameters such as total porosity and mean pore size obtained from these images were satisfactory linked to the mechanical properties of the gels.

Firstly, it may be concluded that the low acyl gellan gum concentration was closely related to the microstructure of the gel networks. As the gellan concentration increases, the strands thickness become thicker and the porosity value become lower and the gel become stronger. These obtained results were consistent with their mechanical behaviours.

Secondly, the salt concentration (as a cross linking agent) play a main role on the microstructure of these gels. Above the critical concentration point an increase in salt concentration led to decrease the porosity value, therefore, the gel becomes more brittle. On the contrary, below the critical point, an increase in salt concentration led to increase the porosity value, thus, the gel becomes more brittle.

Thirdly, the microstructure of the gellan gels can be significantly affected by cyclic compression. As after cyclic compression plenty amount of water expelled throughout the structure of the gellan gels, therefore, its microstructure was significantly altered by cyclic compression. As a result, cyclic compression led to increase the porosity value and increase the thickness of strands, therefore, the gels

become stronger but more brittle. These obtained results were consistent with their mechanical behaviours.

Chapter 6. Release

6.1. Overview

In chapter 4, the effects of the gellan gum concentration, salt concentration and cyclic compression on the mechanical properties of the low acyl gellan gum gels were investigated by using uniaxial compression tests. The purpose of the experiments was to understand the effect of these factors on the mechanical properties of the gellan gels at failure point which can be affected the release from the gels. Chapter 5 described the microstructure of the gellan gels to investigate the effect of gellan and salt concentration, also, the effect of the cyclic compression on the microstructure of the low acyl gellan gum gels.

This chapter focuses on understanding the effect of the low acyl gellan gum concentration, salt concentration and cyclic compression on the flavour release from the gels. This has been achieved by using Instron universal testing machine to release the salt, and riboflavin from the specimens with different gellan and salt concentration following the cyclic compressions. Several release profiles from different gellan gels with different salt and gellan concentrations were obtained, also, the release profile of the gels after cyclic compression were gathered to investigate the effect of cyclic compression on release from the specimens. This chapter will establish, within the release:

- i. The effect of the low acyl gellan gum concentration on salt, and riboflavin release from the gellan gel;

- ii. The effect of the salt (cation) concentration on the salt, and riboflavin release from the gellan gel;
- iii. The effect of the cyclic compression on the flavour release from the gellan gels;

6.2. Introduction

One of the most growing concerns of public health authorities across the world is to diminish the intake of salt in food items. Scientific evidence links major health illness and diseases such as hypertension, cardiovascular diseases caused by stress, cataract, kidney stones, gastric cancer, diabetes and osteoporosis to the daily consumption of salt in our diet (de Loubens et al. 2011) (Elliott & Brown 2006). The ideal intake of sodium chloride on a daily basis as suggested by WHO-World Health Organisation is 5 grams. For certain developed countries, however, the consumption of salt is generally 2-3 times greater than this level. Minimising the content of salt in foods poses quite a challenge for producers of food items as it frequently causes to a loss of organoleptic qualities. Because the structure and texture of food will ultimately influence the aroma and taste perceptions of the consumers, there is little possibility that the food matrix is designed in a manner which reduces the content of salt without diminishing the perception of saltiness ((de Loubens et al. 2011) (Phan et al. 2008) (Saint-Eve et al. 2009) (Koliandris et al. 2010),(M. Panouillé et al. 2011).

Many drug delivery and release systems which utilize natural polymers are based on protein and polysaccharides. Due to their beneficial features for example, non toxicity, biodegradability and biocompatibility, hydro gels from natural polymers, particularly polysaccharides are commonly used (Olteanu et al. 2008).

According to Aguilera (2005), gels are essential products which include puddings, dairy products, textured fruits, processed meat products and their replacers, fish (Surimi) etc. Food gels are a complex mixture of multi-component hydrated biopolymers. The characteristic of such gels is that water is enclosed in the matrix of the gel and provides the gel the distinguishing semi-solid character. There are several polysaccharides which comprise of gellan, alginate, pectin, carrageenan, and proteins like gelatin, soy and whey protein which are excessively being used to produce food gels. (Embuscado et al., 2009; Hammad *et al.*, 2011; Banerjee & Bhattacharya, 2014).

The exceptional semi-solid character of food gels is mainly because of their capability to capture the serum in their structure. Since the serum is the most proportional component, it is possible to be released from the gels during oral processing. This is actually required for items like processed meat products and replacers, where serum releases are necessary to retain the perception of juiciness. The quantity of serum and its characteristics are the most significant indicator for many consumers on the quality of the meat. Conversely, for puddings or related dairy products, the flow of serum during the oral processing is normally considered as a flaw. The ejection of serum is linked with the big deformations during oral processing. Despite this, many studies on mechanical properties of gels, consisting mixed gels have been performed by applying small deformations. Aguilera & Stanley (n.d.), Clark et al., (2002); Cui et al. (2005) did comprehensive studies on the small deformation properties of the gels. Nevertheless, it is important to examine the behaviour of gels under large deformation and cyclic compression, so that it is easy to inter-relate their sensory perception with their mechanical properties.

6.3. Flavour Release

Several studies have been conducted in order to examine the influence of hydrocolloid gels' texture on the flavour release; however, the results were only occasionally coherent. According to Boland (2004), flavour release is majorly influenced by the texture of the gels. Gels having the greatest level of Young's modulus are capable of releasing the smallest quantity of flavour. The basic purpose of the study was to have supplementary examination on the proposition that the dominate flavour release perception is the release of the tastants from the hydrocolloid matrix. In case of gels, it will be controlled by the gel's brittleness.

Several studies have revealed that hydrocolloids affects the rate and intensity of flavour release in foods (Gijs et al. 2000, Cook et al., 2003; Hollowood et al., 2003; Gonza'lez-Toma's et al., 2004; Boland, 2004; Bortnowska, 2005, Seuvre et al. 2006). Many of these studies are actually linked with the impact of the hydrocolloids on flavour perception. Nevertheless, to comprehend these phenomena, there is a need of physic-chemical studies. The impact on perception may be linked to alterations in concentrations of flavour released from the gel system or to the perception of a thickener solution. In addition, evidence shows that all of these three factors that is the thickener system, firmness of the gel and the specific flavour compound used should be considered as they all affect the rate and degree of flavour release (Boland, 2004; Bortnowska, 2005, Seuvre et al. 2006).

6.3.1. Salt Release

Four distinct concentrations of the low acyl gellan gum were used to perform the salt release experiments. For every range of the gellan concentration, three gels with

varying salt concentration were chosen having different rigidity like soft, medium and hard gel, respectively. During the discussion of uniaxial compression and cyclic compression, it was already mentioned in Chapter 4 that the gels expelled significant quantity of serum from the sides. As per the acquired results from Chapter 4, the quantity of serum (water) release matched with both the difference in the mechanical properties as well as the microstructure of the gels subsequent to the compression and cyclic compression. Gels having a greater gellan gum concentration possessed a thicker microstructure in the gel network. This enables the gel to capture the water in the microstructure and, hence, the gellan concentration will influence the release of serum. The increase in the viscosity lead to a decrease in the aroma and taste perception, perhaps binding, mouth coating and fresh surface generation also play a part in its behaviour (Malone *et al.*, 2003).

6.3.1.1. Effect of the Low Acyl Gellan Gum Concentration on the Salt Release

Figure 6.1, Figure 6.2 and Figure 6.3 represent the experiment results of the salt release. It can be noted that for gels with varying gellan concentrations following distinct number of cycles, the gellan gels having 0.6% gellan concentration demonstrate the maximum release of salt. Yet, the gel having 1.2% gellan concentration shows minimum amount of salt release in every range of gellan rigidity. It can be seen that with the increase of gel concentration, the salt release decreases. Relating to the conclusion drawn in Chapter 4, when the gellan concentration is increased, the true stress and energy per unit volume increases, whereas Young's modulus decreases at failure point. With respect to the microstructure of the gels, when gellan concentration increases, the gel becomes stronger. For higher gellan

concentrations, the higher true stress at failure point might be because of greater concentrated gellan network as the same was seen in Chapter 5 that gels having higher gellan concentrations are stronger because of lower porosity and, hence, it can be deduced that the gellan concentration influences the salt release. The gathered conclusion from the experiment shows that an increase in gellan gum concentration will cause a decrease in the release of salt from the gels.

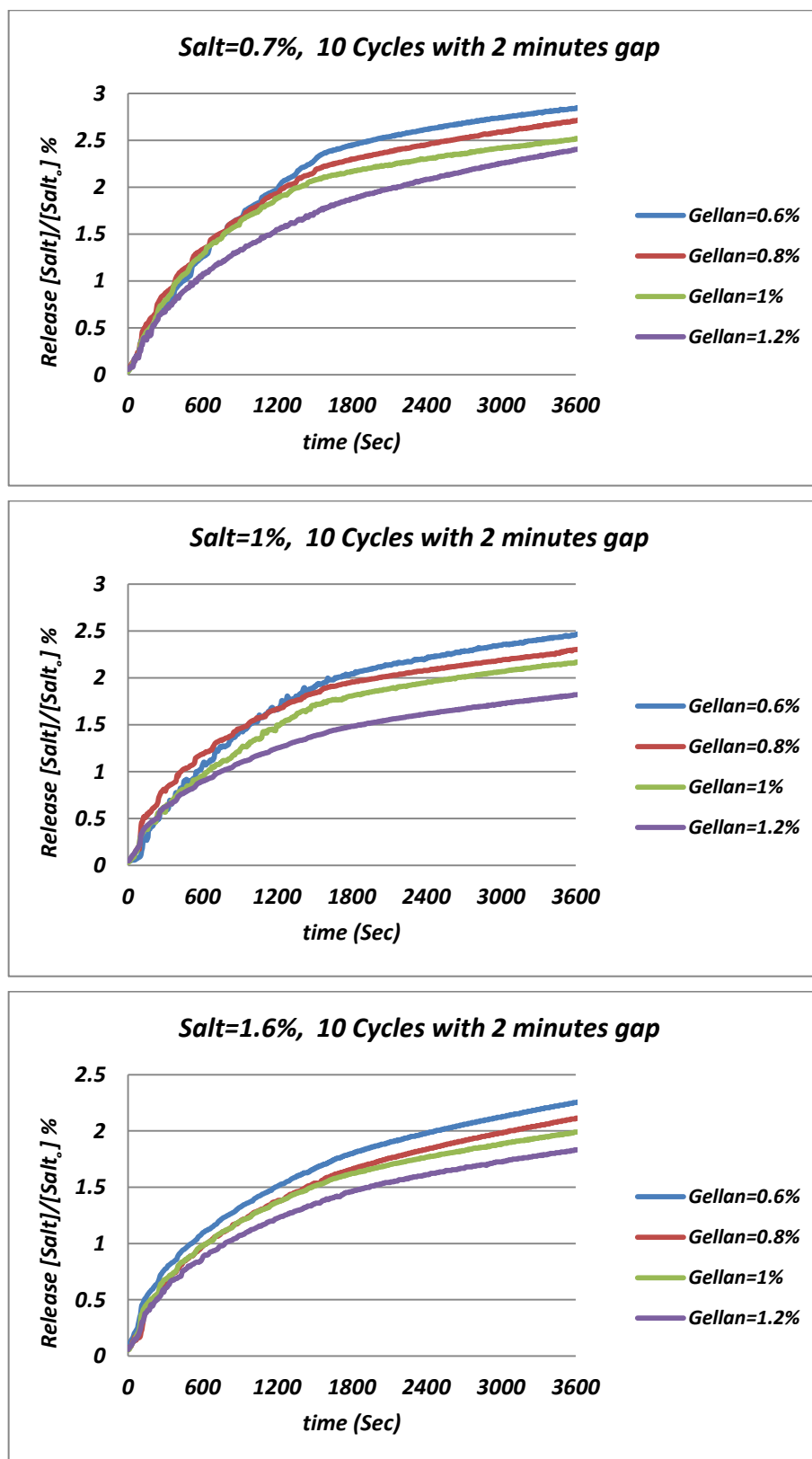


Figure 6.1 The effect of the low acyl gellan gum concentration on salt release from gellan gels following 10 Cycles with 2 minutes gap.

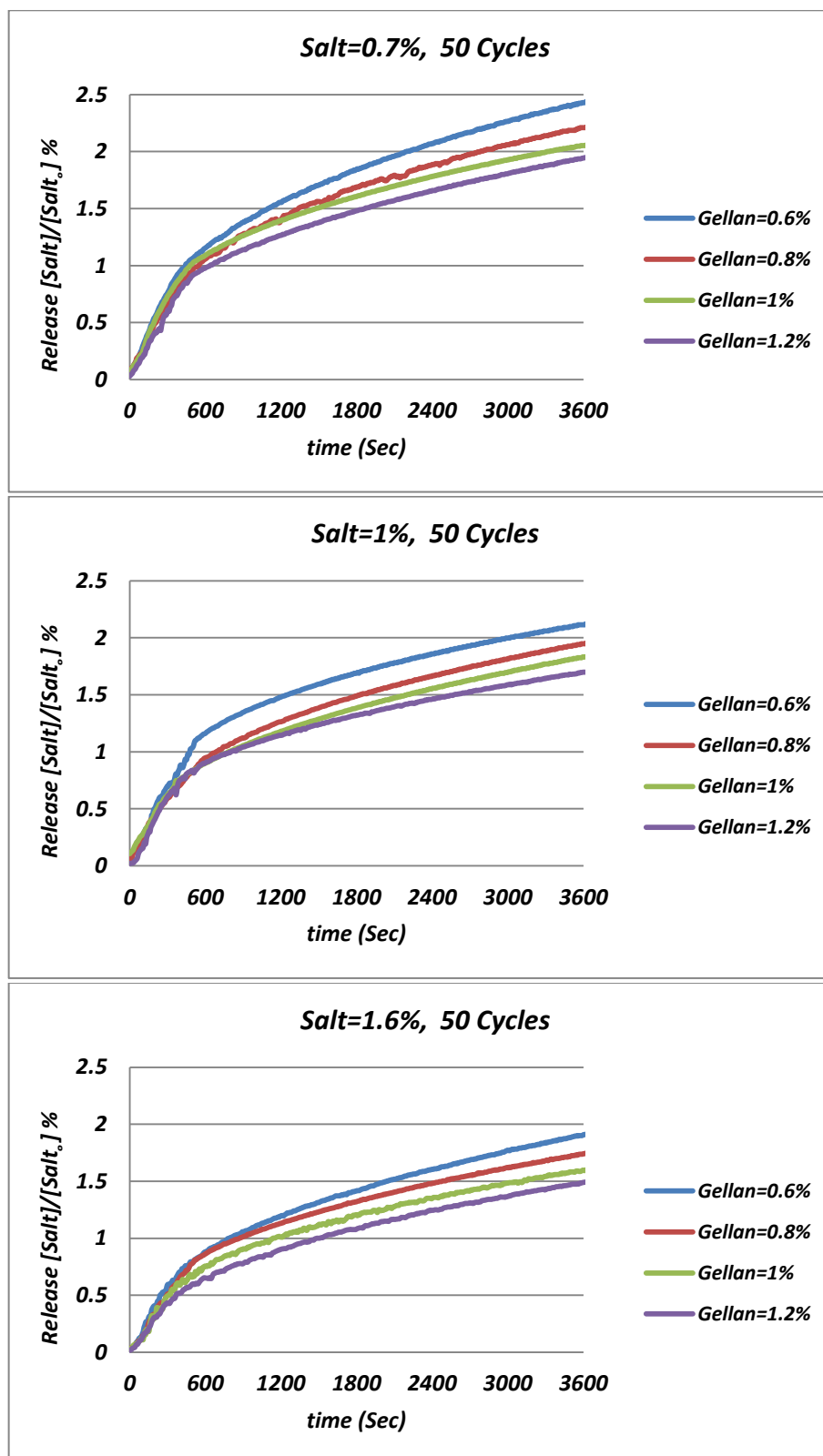


Figure 6.2 The effect of the low acyl gellan gum concentration on salt release from gellan gels following 50 Cycles.

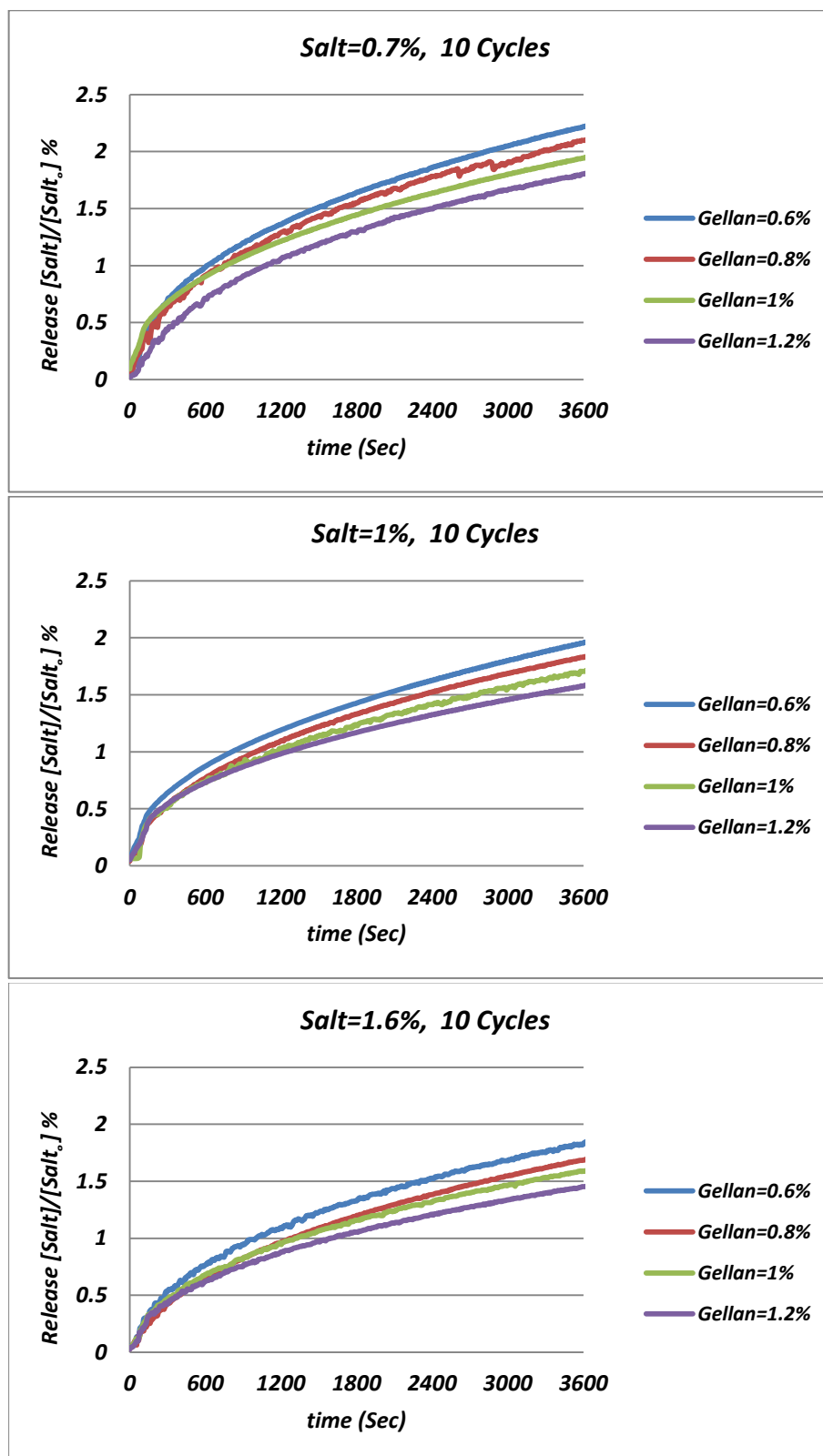


Figure 6.3 The effect of the low acyl gellan gum concentration on salt release from gellan gels following 10 Cycles.

Figure 6.1, Figure 6.2 and Figure 6.3 depict the release of salt from the gels samples at ambient temperature. For every sample, a comparison is made between the gels with varying gellan concentrations used at a specific salt concentration. It can be seen that by increasing the gellan concentration, a decrease in the salt release will occur. During comparison of a single concentration curve for each of the gellan concentration levels, the pattern of salt release is the same. Between each gellan concentration, certain variation on release was examined which points towards the fact that increasing low acyl gellan gum concentration causes a reduction in the salt release from the gels because of the increased physical interference from gellan chains inside the structure.

As already discussed in Chapter 4, the mechanical properties of the gellan gels is influenced by the concentration of the gellan. When the gellan concentration is increased, the gel tends to be stronger and harder because of the elevated level of stress at failure point. Hence, when the gellan concentration is increased, the salt release is decreased. Chapter 4 already covered that hard gels need more energy per unit volume at failure point; hence, any increase in gellan gum concentration will cause an increase in the energy per unit volume at failure point. Therefore, the gels need additional energy to rupture, so that the serum is released. The derived conclusions are in accordance with previous studies. Other studies on the same indicated that firm gels release flavour with lower maximum intensity as compared to soft or medium gels (Boland 2004) (Guinard & Marty 1995). Brittle gels were seen to release more salt during a two-bite compression as compared to soft and elastic ones (Koliandris 2008b). Koliandris and co-workers demonstrated that fracture strain was inversely correlated with salt release.

There are 2 mechanisms which can explain the impact of hydrocolloids on flavour release. The first one is the physical entrapment of flavour molecules inside the food matrix. Research carried out by Huang and Brazel (2001) proved that the existence of an entangled polymer network hinders the movement of small molecules like for instance flavour from inside the gel system to the surface. The other mechanism entails interaction between the flavour molecules and the gel components (Arvisenet et al., 2002; Naknean & Meenune, 2010). Breakdown of food resulted in the release of flavour volatiles as well as the surface area available for diffusion. Thus higher breaking strengths and lower perceived flavour intensities were experienced by the harder gelatine gels, states (Hollowood, 2002) (Wilson & Brown, 1997).

6.3.1.2. Effect of the Salt Concentration on the Salt Release

This particular section deals with salt release from gellan gels structure with different salt concentrations. The experiment was performed to examine the influence of the salt (cation) concentration as a cross linking agent on salt release. Initial experiments were conducted out on the 3 gellan gels with varying rigidity (soft, medium and hard). When comparing soft or medium gels, the firm gels released flavours with lower maximum intensity states (Boland, 2004). Gellan samples, at different salt concentrations were combined in the vessel of distilled water and, meanwhile, the conductivity was recorded to track the salt release, as explained in materials and methods chapter.

The mechanical properties and microstructure of the gels affect the salt release. With respect to the attained results from prior chapters, the rigidity and strength of the gels can be influenced by the salt concentration as the salt holds a significant place in the microstructure of the gels as a cross linking agent. To examine the influence of the salt (cation) concentration on the salt release, 3 gels with varying range of microstructure were chosen like soft, medium and hard, respectively. With respect to the acquired results from Chapter 4, the mechanical properties of the gellan gels like hardness, strength and brittleness can be influenced by the salt (cation) concentration. It was noted that gels having greater true strain but lower strain and lower energy per unit volume were more brittle. Because of the brittleness of the gels, some micro cracks will appear on the gels structure after the compression, specifically cyclic compression. Hence, the release from the gels will increased due to the micro cracks on the gels structure.

Figures 6.4, 6.5, 6.6 and 6.7 represent the findings from the salt release experiments for gels with varying salt concentrations. It was seen that gels with varying rigidity displayed disparate salt release. The salt release results for three types of gellan gels as hard, medium and soft are shown in Figure 6.4, 6.5, 6.6 and 6.7 respectively. It can be seen that the gels having 0.7% salt as a hard gel display the maximum salt release, but the other gels with more salt content and less rigidity display a diminished quantity of salt release because of the decrease in the brittleness subsequent to the modifications in the microstructure and mechanical properties of the gels following a change in the salt content. The finding of the present study is in line with the previous study where more salt was released by the brittle gelatine gel during the two bite compression as compared to the soft elastic ones (Koliandris *et al.*, 2008). There exist an inverse relationship between the fracture strain and the salt release. Thus salt release would increase to a greater extent owing to the breakdown of product and an increasing contact area between water and product, although the strain level was higher than the failure strain salt release (de Loubens *et al.*, 2011).

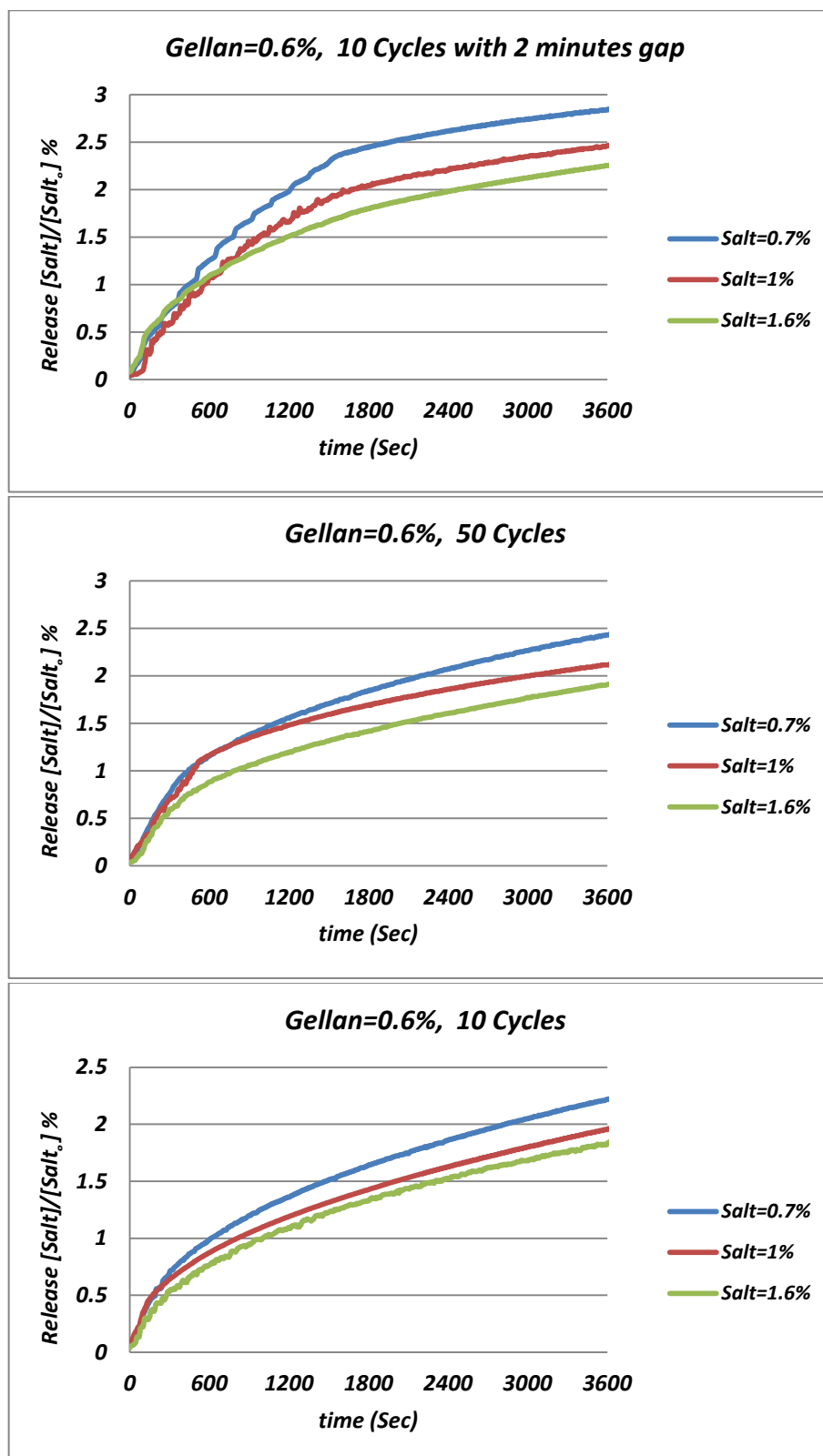


Figure 6.4 The effect of the salt concentration on salt release from 0.6% gellan concentration gels following 10 Cycles with 2 minutes gap, 50 cycles and 10 cycles, respectively.

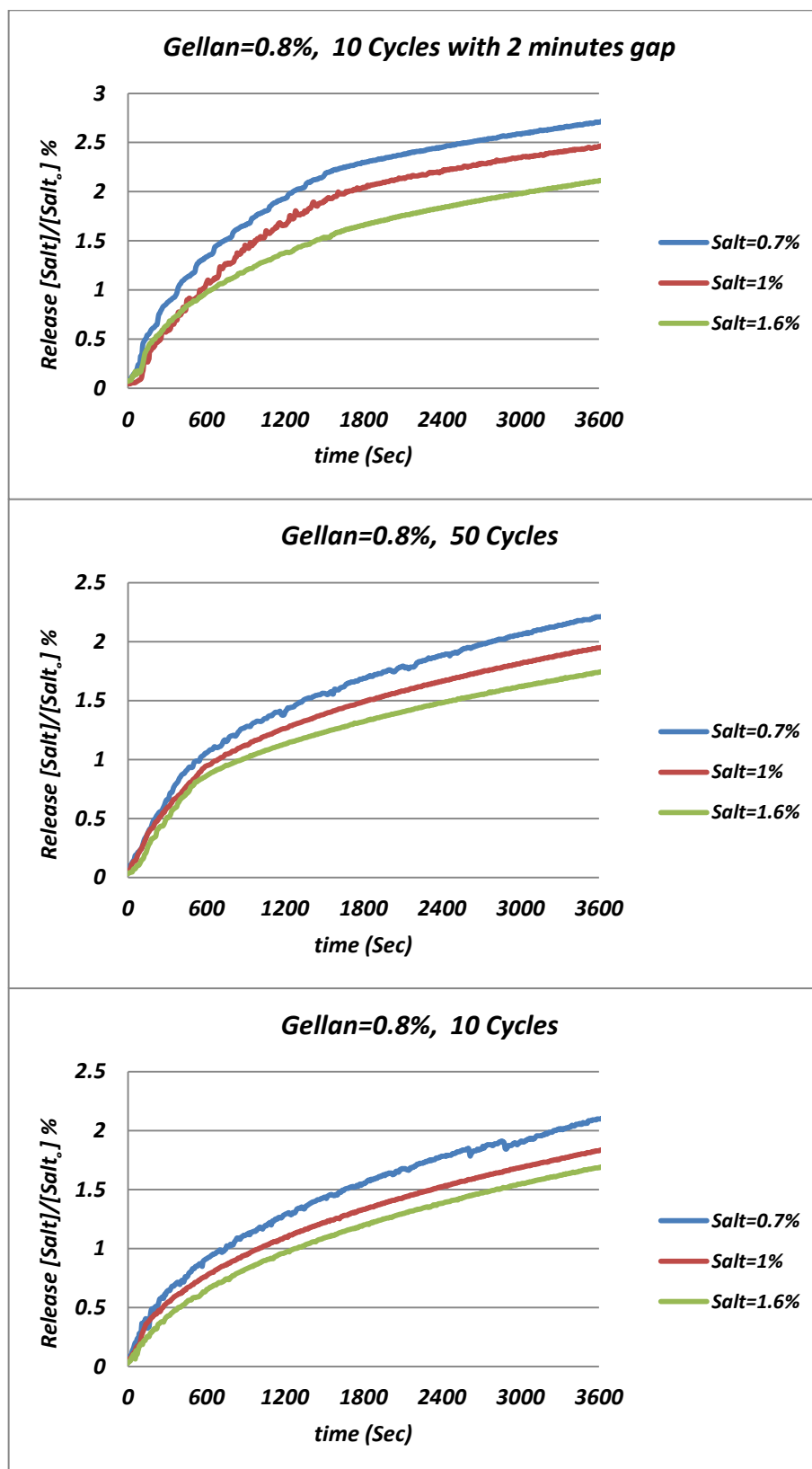


Figure 6.5 The effect of the salt concentration on salt release from 0.8% gellan concentration gels following 10 Cycles with 2 minutes gap, 50 cycles and 10 cycles, respectively.

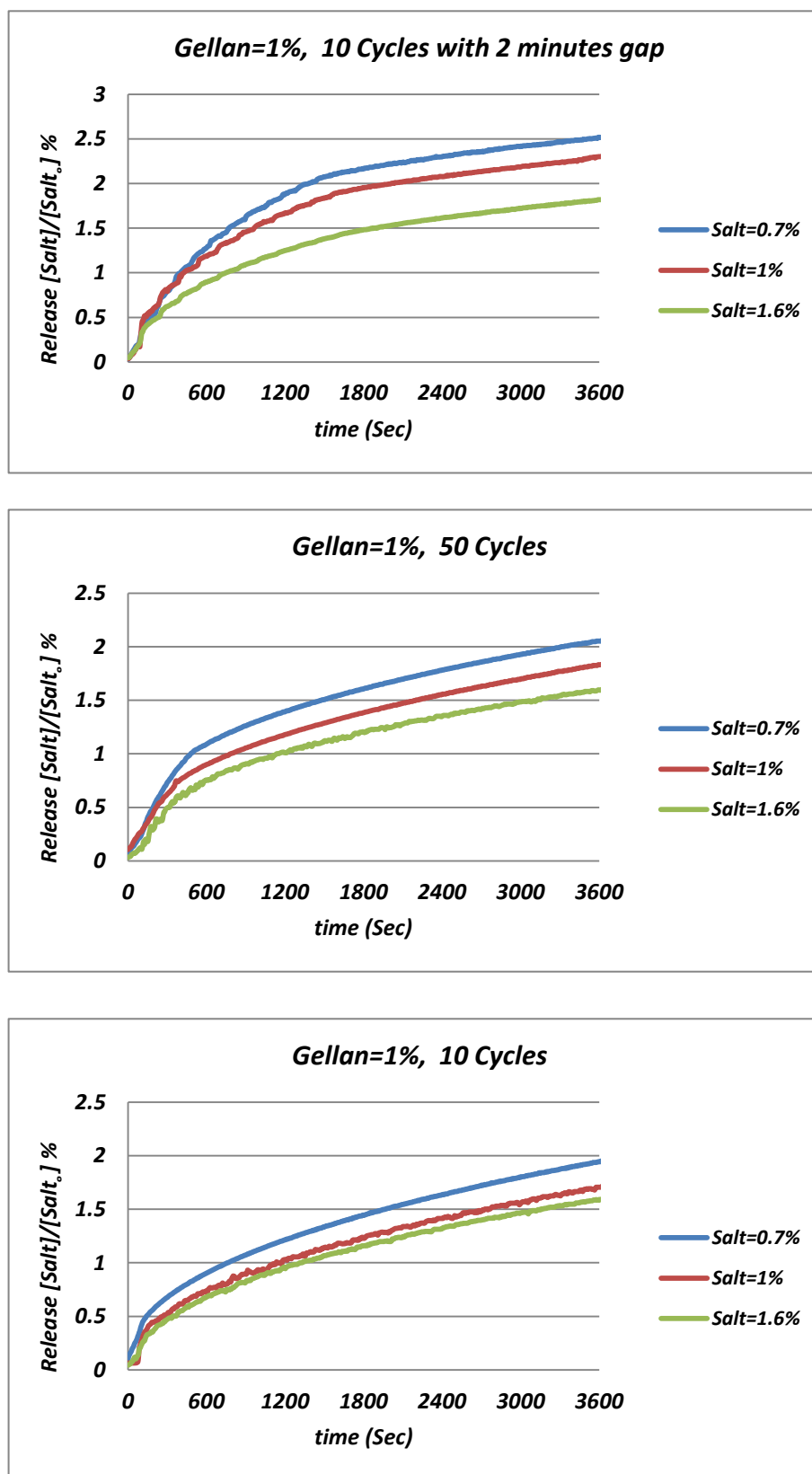


Figure 6.6 The effect of the salt concentration on salt release from 1% gellan concentration gels following 10 Cycles with 2 minutes gap, 50 cycles and 10 cycles, respectively.

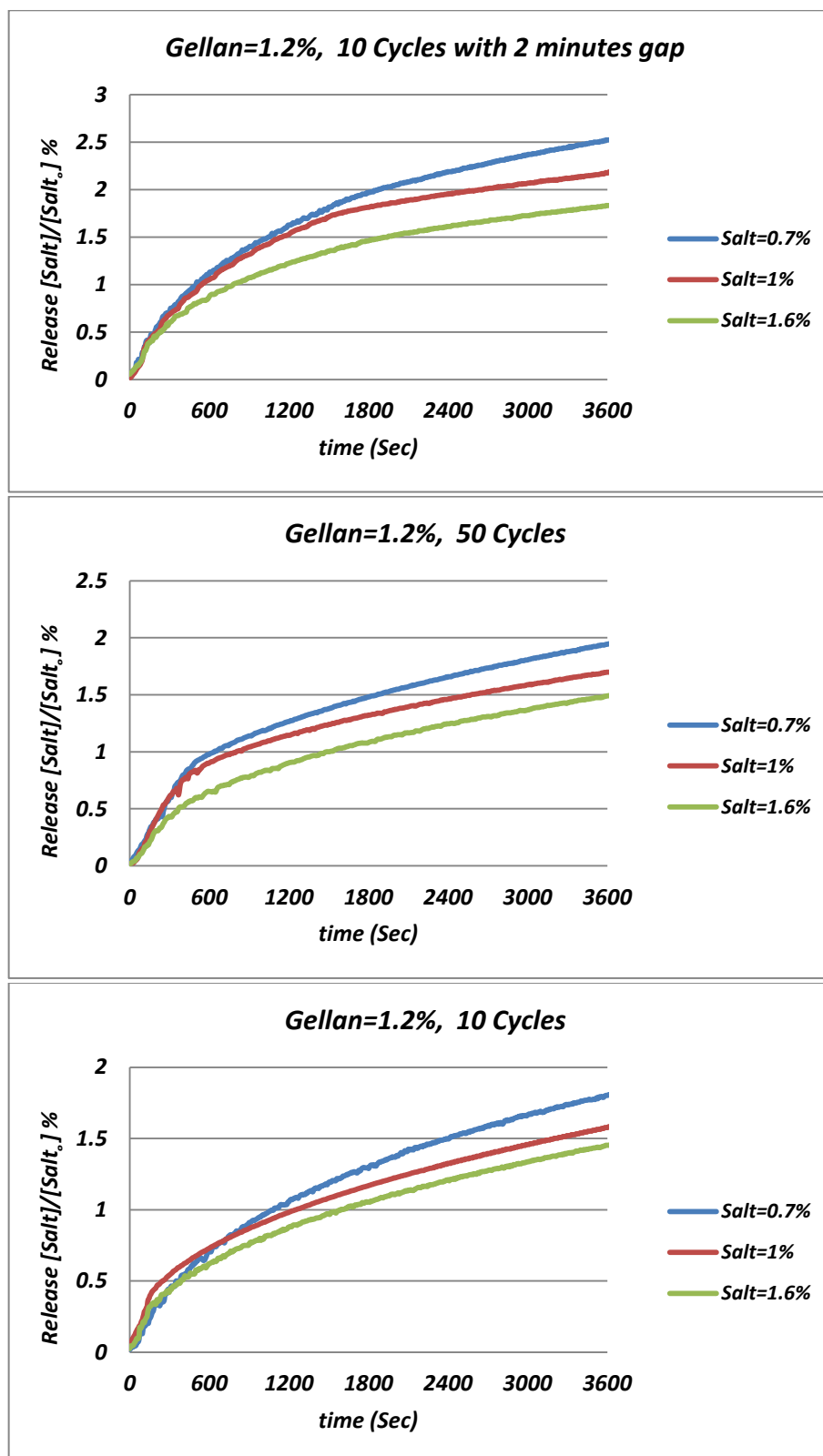


Figure 6.7 The effect of the salt concentration on salt release from 1.2% gellan concentration gels following 10 Cycles with 2 minutes gap, 50 cycles and 10 cycles, respectively.

For other gellan gels with varying gellan concentrations, a similar trend was observed. The achieved findings correspond with the results from prior studies. Experiment conducted by Mills et al. (2011) demonstrated that the gel's structure will identify the change in release. If the gels are brittle then the salt is released faster as compressions cause higher break up.

In the gellan gels, water flows out from the gel after they are compressed (Harris, Smith et al., 2008). Once the water is expelled after compression and cyclic compression, it is expected that salt will be released at a quicker pace. According to Tang et al. (1996), for gellan gels, the mechanical properties were influenced by the cation concentration existing around a critical concentration. The gels were less brittle and have minimized strength, above this critical concentration.

Thus, it can be It can be concluded that the mechanical properties and microstructure of the gellan gels are possible to be influenced by the salt concentration (cation) as a cross linking agent. This is because the mechanical properties and microstructure of the gellan gels have a significant place on the release from the gel. The gels become less brittle above the critical salt concentration (0.7%) and, hence, by increasing the salt concentration, an overall decrease in salt release from the gellan gels is observed.

6.3.1.3. Effect of the Number of Cyclic Compression on the Salt Release

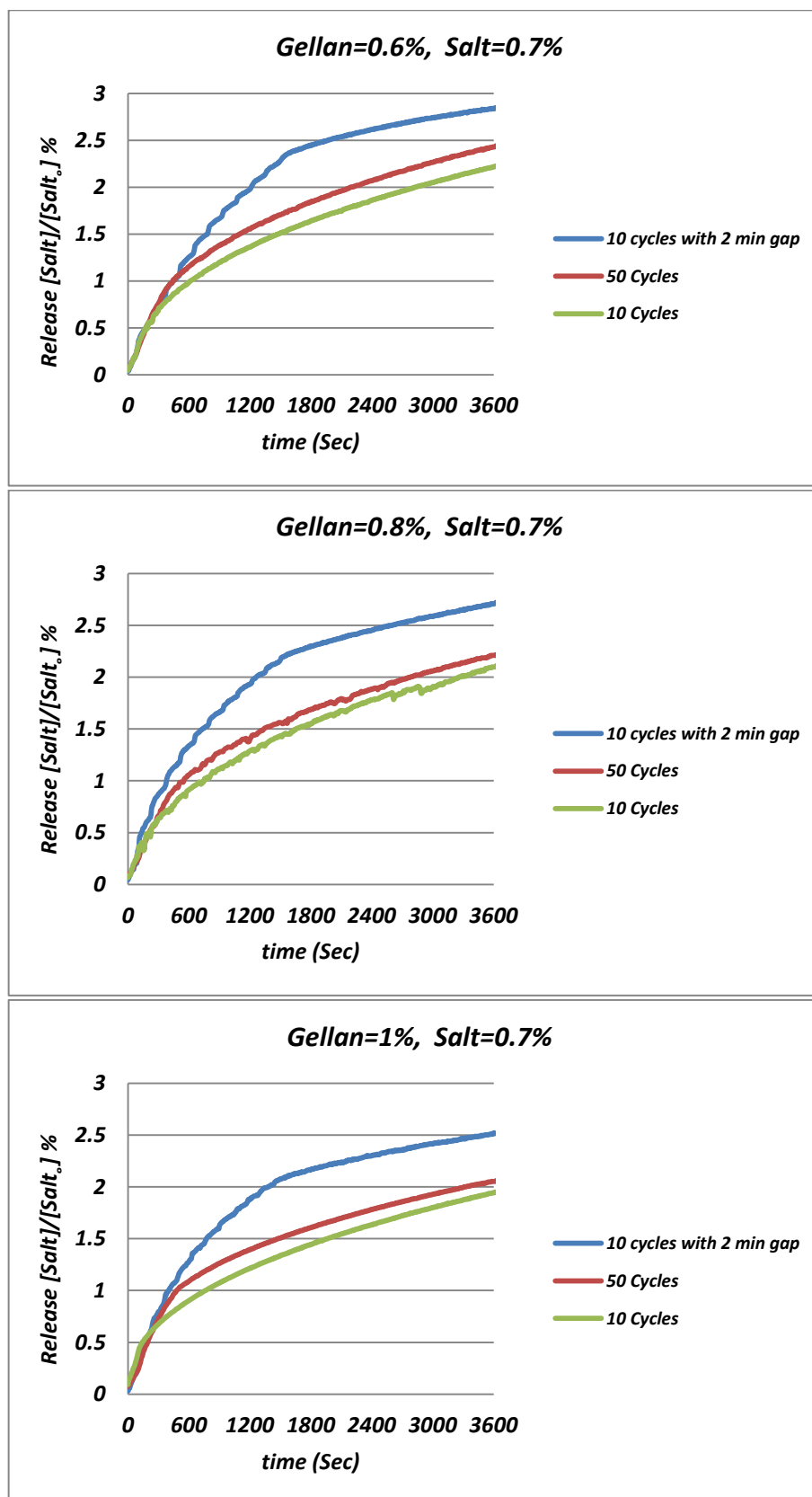
The mechanical properties and microstructure of the gellan gels are influenced by the cyclic compression as explained in Chapter 4 and 5. Hence, it should be noted that cyclic compression holds a significant role on the release from the gellan gels .The

serum release from the gellan gels during cyclic compression is affected by a series of factors like permeability of the gels, force acting on the serum and changes therein during cyclic compression. Liquid flow through a porous material is explained by the Darcy relation (Walstra 2002).

$$Q = \frac{B \cdot A_c \Delta p}{\eta l} \quad (6.1)$$

where (Q) is the volume flow rate of serum permeating through the permeable area (A_c), (B) is the permeability coefficient, (Δp) is the pressure difference acting on the liquid over a distance (l) and (η) the viscosity of the liquid. The permeable area is the outside surface of the gels. The external surface of the gels is actually the permeable area. For the sake of experiment, it is presumed that from the sides above and beneath the gels, serum release does not occur. Compared with water, the viscosity of the serum is quite similar and remains same for samples. The degree of compression will influence the other terms and will surely affect the release of serum. As the deformation increases, the stress term also rises. Therefore, during cyclic compression and deformation, the quantity of serum release will increase. But other terms, like porosity and the permeable area will alter at greater deformation and cyclic compression. The porosity of the gel and the permeable areas, both, will witness a considerable increase as deformation and cyclic compression increases. Consequently, certain pores will shrink in size which in turn will decrease the release of serum. The same has also been established from results gathered from prior chapters.

It is quite evident that both serum as well as salt release is influenced by cyclic compression and quantity of cycles. A greater quantity of serum release was noted during cyclic compression at increased number of cycles. This is due to the fact that longer time was required for serum to flow out of the sample. In addition, it can be seen that in the case of 10 cycles with 2 minutes gap in between every cycle, there is a major difference in salt release and other cyclic results. Again this is due to the longer time required for serum to release and expel from the gels between every cycle during cyclic compression.



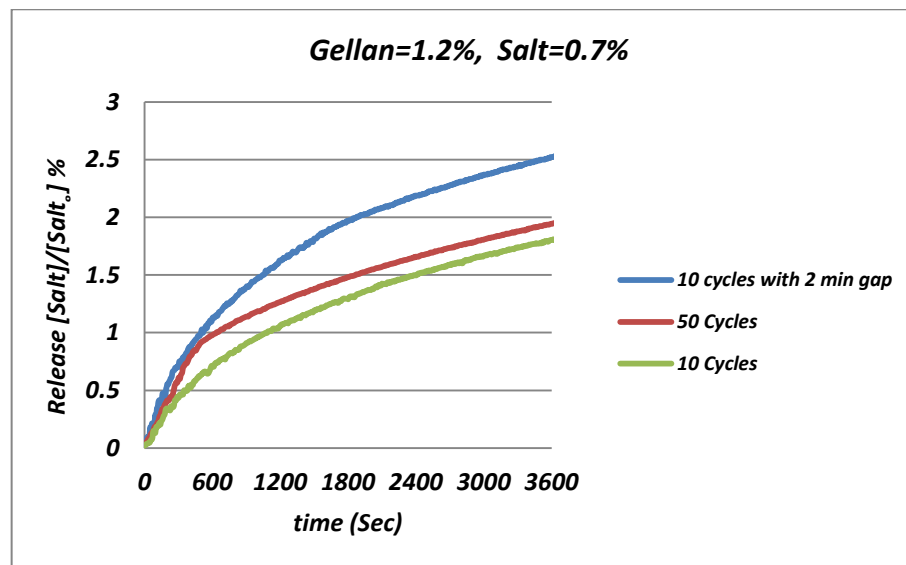


Figure 6.8 Effect of the number of cycles on salt release from the gellan gels with different gellan concentrations.

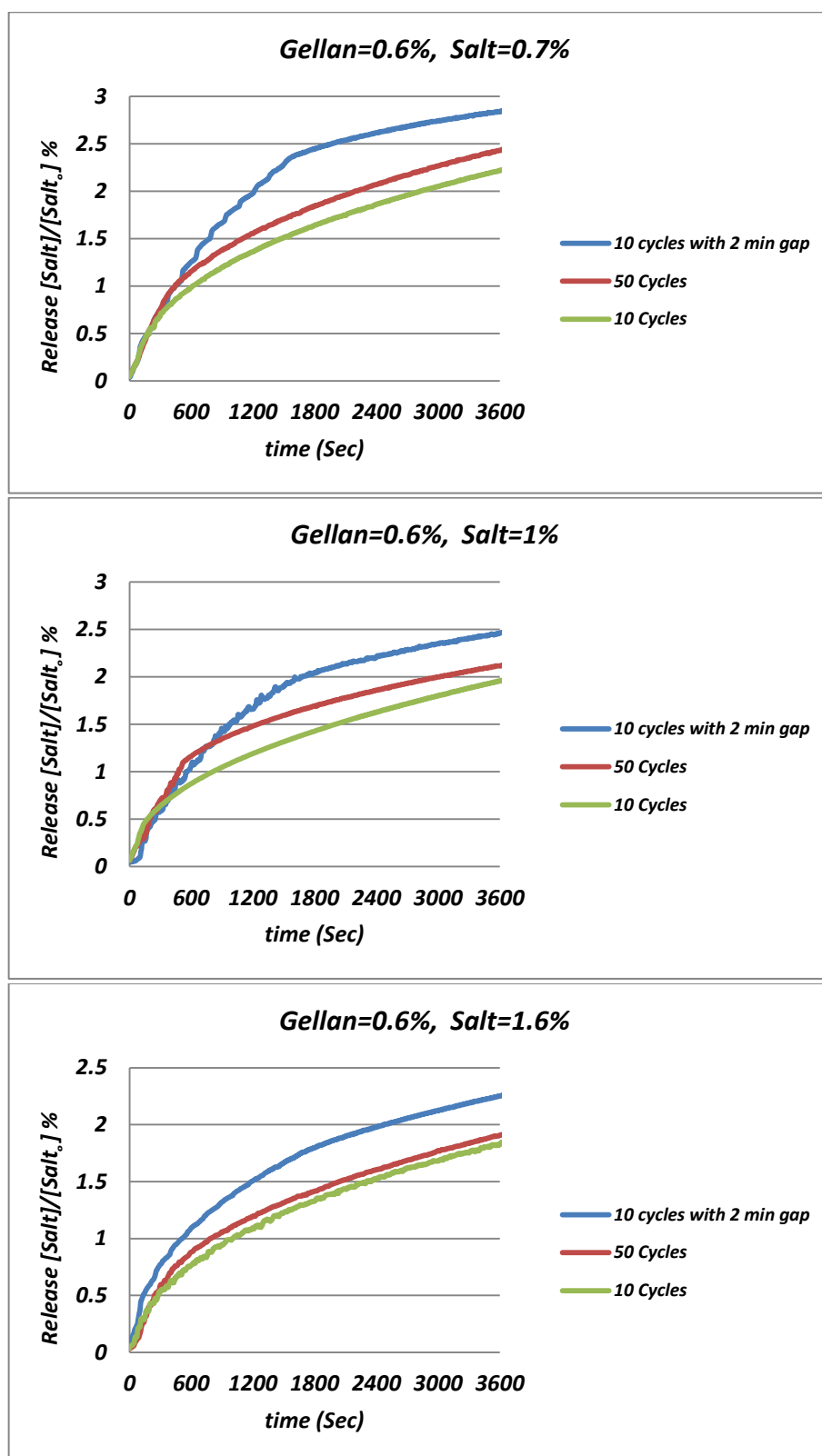


Figure 6.9 The effect of the number of cycles on salt release from the gellan gels with different salt concentrations.

The findings gathered from the experiments were in accordance with the hypothesis that the salt and serum release is affected by the factors involved in Darcy relation: the stress and the porosity along with the permeable area. The balance between the stresses required flow of serum determines the serum flow. The permeable area will affect this balance and is associated with all the side walls of the gels (van den Berg *et al.*, 2007). The stress which flows after the cyclic compression increases and, as a consequence, the flow rate of serum also increases. Nevertheless, as per the results achieved from Chapter 5, subsequent to the cyclic compression, the porosity as the permeable area starts to increase and, later, it causes a change in the release of salt and serum through the gellan gels structure. Figures 6.8 and 6.9 show that as the number of cycles is increased, the quantity of salt and serum release also increases. Hence, it can be concluded that after the cyclic compression, the microstructure and mechanical properties of the gels are changed. With respect to the results achieved from Chapters 4 and 5, the cyclic compression caused the porosity of the gels to increase and, thus, the gels become more brittle. Furthermore, subsequent to the cyclic compression the true stress at failure point is increased, however, the energy per unit volume at failure point and the Young's modulus is decreased. Hence, the gels turn out to be stronger but more brittle. These brittle gels are capable of releasing easier and greater than the other gels. As already mentioned earlier, because of the major serum release during cyclic compression, the salt release throughout the gellan structure is increased.

6.3.2. Riboflavin Release

The release of riboflavin from the gellan gel under cyclic compression is performed in this section. The riboflavin release from gellan gels was conducted in order to

examine the release of a material in absence of any interaction with the gel structure. The objective of choosing Riboflavin was to examine the impact of contact between the gel structure and releasable matters on the release profile from the gellan gels.

Alike the section on salt release, gellan gels with different levels of rigidity were chosen like for example, hard, medium and soft. The samples of gellan gel with 0.1% of riboflavin, consisting of different gellan concentrations were put in the vessel filled with distilled water. During this process, the riboflavin concentration was measured to pursue the release of riboflavin, as explained in the materials and methods chapter.

6.3.2.1. Effect of the Low Acyl Gellan Gum Concentration on the Riboflavin Release

The riboflavin release profile was collected to examine the impact of low acyl gellan gum gel on the riboflavin release. Gels with varying gellan concentrations having riboflavin were subjected to uniaxial cyclic compression. The eventual release of riboflavin from the samples of gellan gel under ambient temperature is depicted in Figure 6.10. A comparison is made for every sample between the gels with varying gellan concentrations at a particular level of salt concentration and different number of cycles.

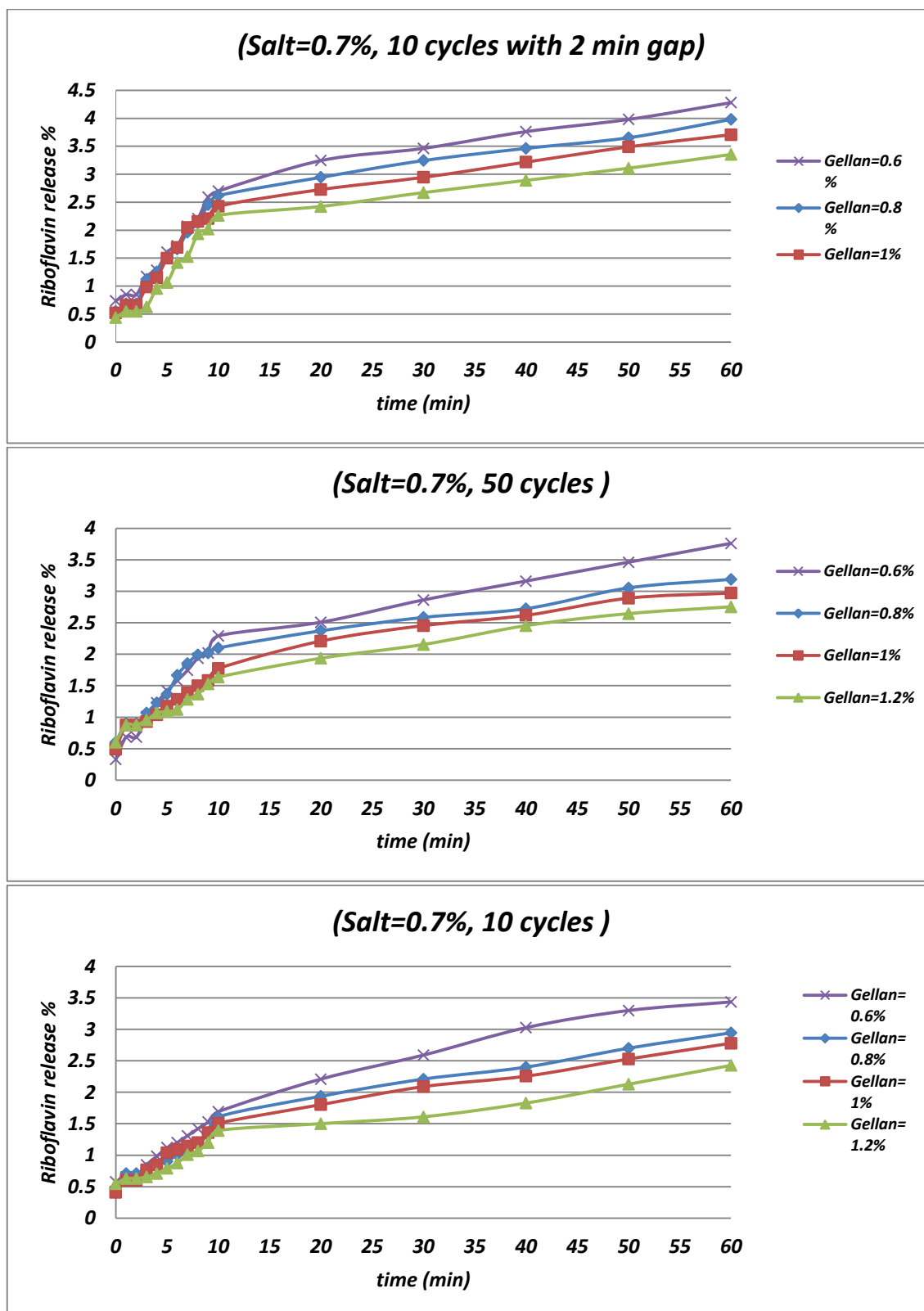


Figure 6.10 The effect of the low acyl gellan gum concentration on riboflavin release from gellan gels following 10 Cycles with 2 minutes gap, 50 cycles and 10 cycles, respectively.

The mechanical characteristics, texture and microstructure of the gels majorly influenced the release from the low acyl gellan gum gels. The gathered results displayed different quantity of salt and riboflavin release for gels having similar gellan and salt concentrations. This can be due to the interactions between gellan-salt and gellan-riboflavin.

The riboflavin release trend corresponds to the collected results for the salt release. As witnessed, there was a similar pattern of riboflavin release and salt release from the gellan gels. A decrease in riboflavin release was observed when the gellan concentration was increased. Nevertheless, the absolute riboflavin release is greater in comparison to the salt release. This will cause the riboflavin release to diminish for gels having greater gellan concentration. The strength and energy/unit volume increases while consequently the Young's modulus of the gels decreases when the gellan concentration is increased. Hence, the gel appears as less brittle and ultimately the riboflavin release through the gel structure can be decreased.

The gathered results indicate that the absolute riboflavin release is greater in comparison to the salt release as there is no interaction between the riboflavin and gellan gum structure. Hence, the riboflavin can be released much easily as compared to the salt from the gel structure. Comparing riboflavin with the salt, a higher overall release was noted for the former. The deduced results are in accordance with previous other studies. Researchers like Weel et al. (2002) and Delahunty et al. (2004) Tyapkova et al., (2013) examined that as the rigidity of the gels is increased, the flavour release is reduced. Meanwhile, Boland (2004) explained that solid and strong gels release flavour with lower maximum intensity as compared to soft or

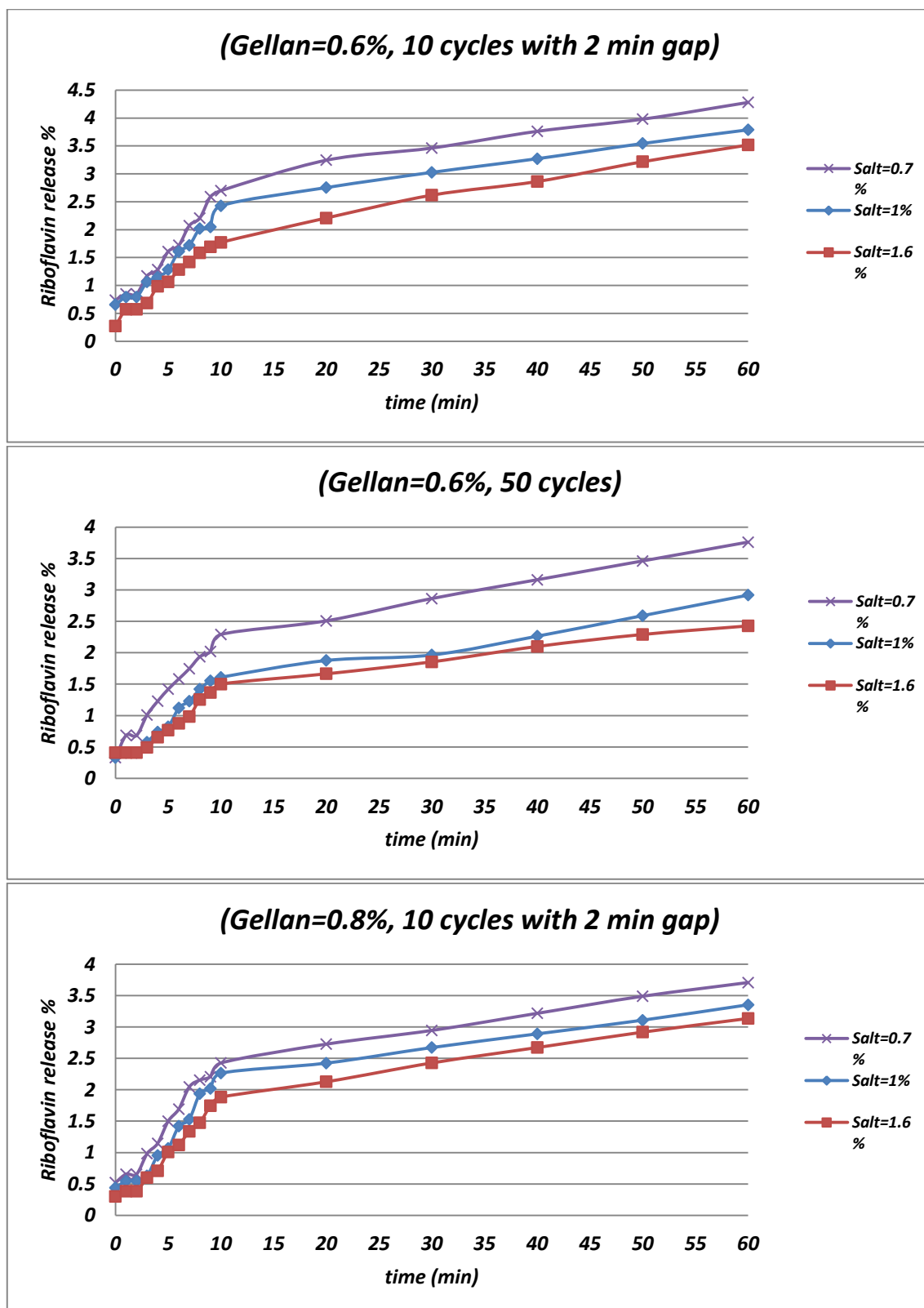
medium gels. Prior studies indicate that the physical entrapment of flavour molecules inside the food matrix is important for the release of flavour from hydrocolloid gels (Boland 2004). In addition, Baines & Morris (1987) observed that presence of an entangled polymer network restricts the transmission of small molecules, like flavour from inside the gel system to the surface. The interaction between the flavour molecules and the components of the gel will influence the flavour release from inside the gels (Mälkki et al. 1993, Boland , 2004). Since there is no interacting or joining capacity of riboflavin to low acyl gellan gel, hence it can be deduced that it is much easy to release riboflavin than the salt from the low acyl gellan gum gels.

It is imperative to mention here that riboflavin's nature is rather amphoteric due to which it has a small net charge and has no interaction with ionic areas in the gellan gel structure (Abd El-Ghaffar et al. 2012). Furthermore, the active groups in low acyl gellan gum chains are basically involved with Na in the formation of cross links. Consequently, it is seen that the weak riboflavin –gellan interactions causes faster and greater amount of riboflavin to be released as compared to salt through the gellan gels.

6.3.2.2. Effect of the Salt Concentration on the Riboflavin Release

Because of the impact of salt concentration on mechanical properties and microstructure of the gellan gel, the salt concentration holds a significant position for the release of riboflavin from the gellan gels. The influence of the salt concentration on the riboflavin release can be examined by collecting the riboflavin release profile from the gels with varying salt concentrations having different levels of rigidity like hard, medium and soft, respectively. Uniaxial cyclic compression was conducted on

the gels having 0.1% riboflavin with specific gellan concentrations and varying salt concentrations. The release of riboflavin from the gellan gel samples over a period of time under ambient temperature is shown in Figure 6.11. A comparison of riboflavin release is made for every sample between the gels with varying salt concentrations at a specific gellan concentration. Chapter 4 has already discussed in detail that 3 gels having different salt concentrations were chosen as hard, medium and soft.



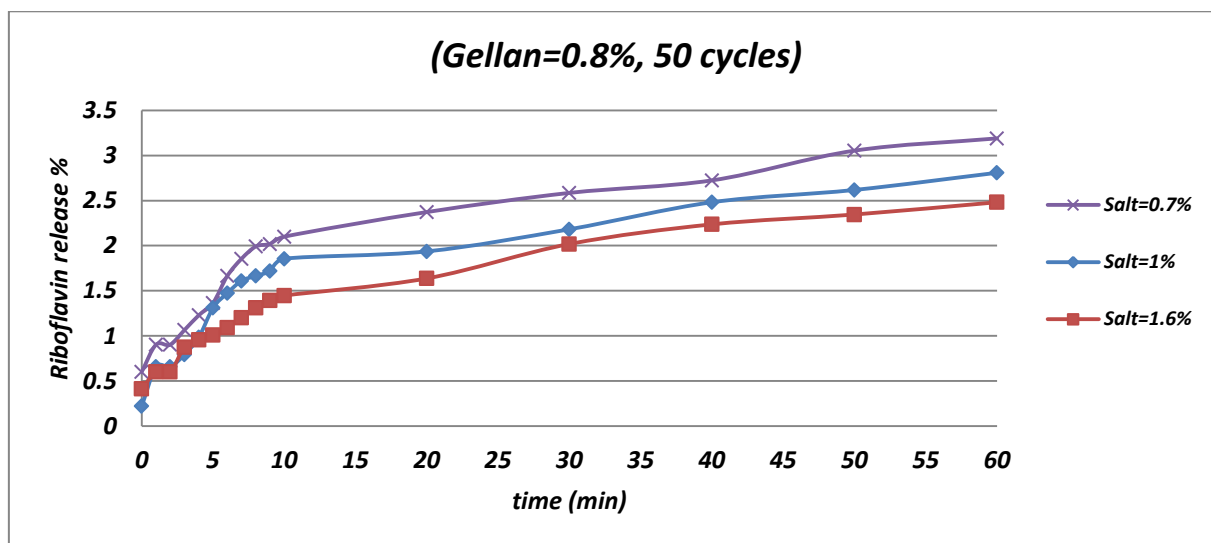


Figure 6.11 The effect of the salt concentration on riboflavin release from gellan gels with different gellan concentration and following different number of cycles, respectively.

The materials and methods section explains the testing of the gellan gel samples. The tests were carried out on samples of the gels having varying low acyl gellan gum concentrations. The release of riboflavin, on the other hand, was followed by off line riboflavin measurement (more details on the same have been discussed in materials and methods chapter). Cyclic compression tests were performed to examine the impact of salt concentration on riboflavin release.

According to Tang (1996), for gellan gels the mechanical properties are influenced by the cation concentration as a cross linking agent existing around a critical concentration. The gel appears less brittle and had smaller strength above this critical concentration.

It was noted that the pattern of riboflavin increase was similar to the release of salt from gellan gels and also that riboflavin release trend is parallel to the deduced findings for the salt release. By increasing the salt concentration at a level above the

critical salt concentration, a decrease in the riboflavin release is observed. Yet, the absolute riboflavin release is greater in comparison to the salt release. The riboflavin release for gels with greater salt concentration is expected to diminish above the critical salt concentration. The increase in salt concentration above the critical concentrations will cause the strength to diminish and the energy per unit volume and Young's modulus of the gels to increase. Once the gels will be less brittle, it is possible to diminish the release through the gel structure.

Greatest release of riboflavin was shown by gels with 0.7% salt concentration (hard gel) at critical salt concentration. Subsequent to the increase in salt concentration, a decrease in the release of riboflavin was observed for medium and soft gels. Above the critical salt (cation) concentration, an increase in salt concentration as a cross linking agent will make the gels less brittle and thus, the riboflavin release accordingly decreases.

6.3.2.3. Effect of the Number of Cyclic Compression on the Riboflavin Release

The findings collected from the experiment of salt release indicate that cyclic compression certainly influences the release of salt from the gellan gels. The riboflavin release from gellan gels is influenced by cyclic compression as the process of cyclic compression causes changes on both the mechanical properties as well as the microstructure of gellan gels.

Subsequent to the cyclic compression, the riboflavin release from the gellan gels is compared in Figure 6.12. As presumed, there is a similarity in riboflavin and salt release trend. An increase in the number of cycles will also increase the riboflavin

increase. The release of riboflavin after ten cycles with two minutes' gap in between every cycle depicts the release of riboflavin at its maximum. This corresponds to the achieved findings from the release of salt within the range of specific cyclic styles. Therefore, it can be easily guessed that the time required for the serum to flow out of the sample was longer for ten cycles with two minutes gap between each cycle as compared to the other cyclic compression styles. This causes the quantity of riboflavin and serum release to increase.

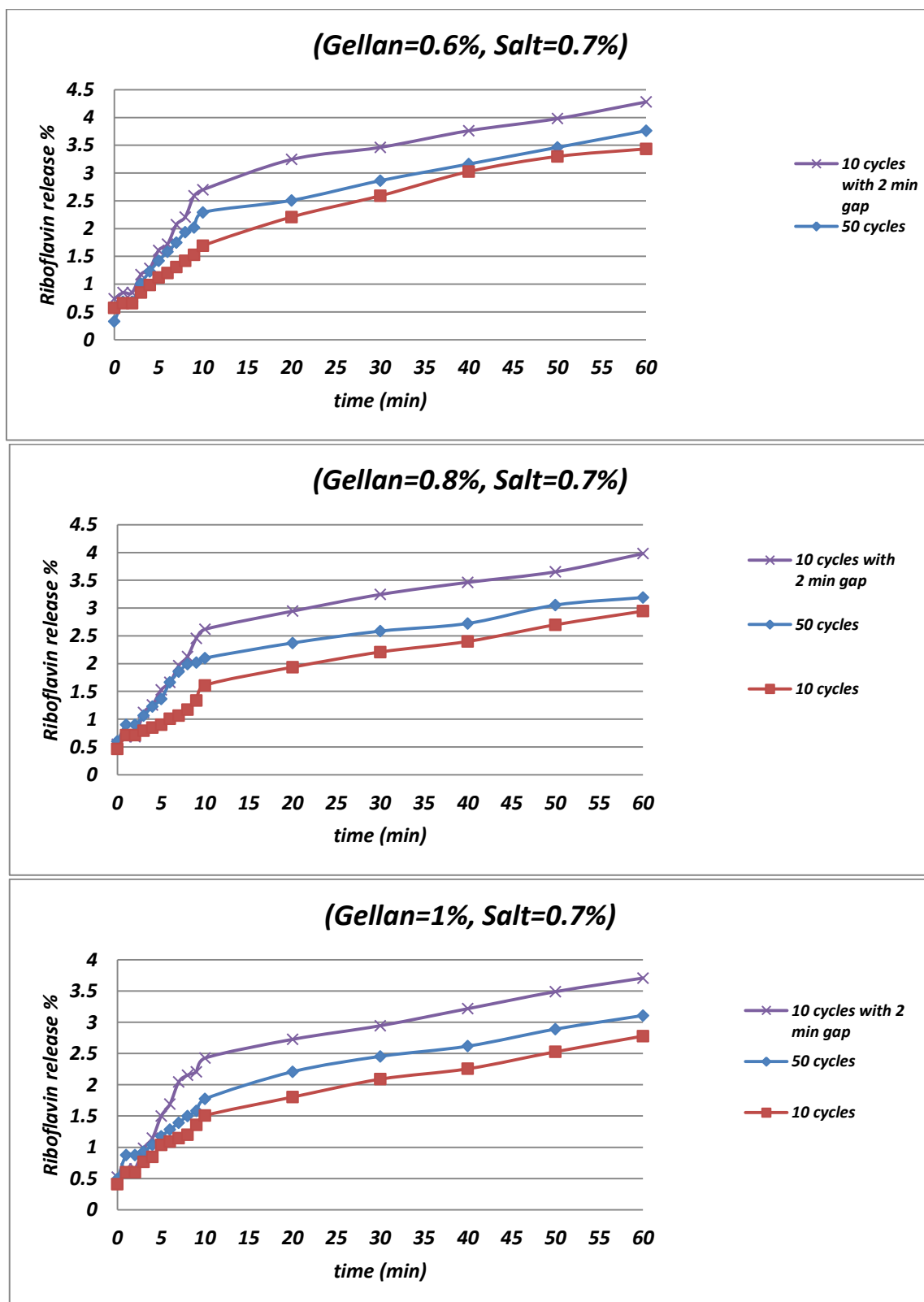


Figure 6.12 The effect of the number of cycles on riboflavin release from gellan gels with different gellan concentration.

6.4. Conclusion

The subject chapter concentrated mainly on the release of salt and riboflavin from the low acyl gellan gum gels. Amongst the important evaluations, the release experiments considered the impact of:

- Low acyl gellan gum concentration,
- Salt (cation) concentration (as a cross linking agent), and
- The cyclic compression on salt and riboflavin release from the gellan gels.

The first conclusion derived from the experiment is that the release is possibly affected by the low acyl gellan gum concentration. The achieved results demonstrated that increase the gellan concentration will cause the release from the gellan gels to decrease because of the reduced brittleness of the gels. When gels have the greatest brittleness, the release was noted to be greatest. Gels which appear to be more brittle will also release more in comparison to other gels. These results achieved from the study correspond with their mechanical properties and microstructure of the gellan gels.

The second observation is that another important factor on the release from the gellan gels is of salt concentration as across linking agent. At a point which is above the critical salt concentration, any increase in salt concentration will cause the release to decrease. With this increase in salt concentration above the critical point concentration, the brittleness of the gels will diminish and hence the release from the gellan gels is reduced. These results achieved from the study correspond with their mechanical properties and microstructure of the gellan gels.

Thirdly, the release from the gellan gels can be affected by cyclic compression. During cyclic compression at higher number of cycles resulted in significantly higher serum release because the time for serum to flow out of the sample was longer. Also, as it can be observed a significant difference in release is between the case of 10 cycles with 2 minutes gap between each cycle and the other cyclic results, because of the longer time for serum to release and flow out from the gels.

The third observation is that the cyclic compression will influence the release from the gellan gels. In the process of cyclic compression at greater number of cycles, a great quantity of serum is released. This is due to the fact that the time for serum to flow out of the sample was longer. Furthermore, it was also noted that between ten cycles with two minutes gap in between every cycle, there is a considerable difference in release and the other cyclic findings. It is due to the prolong time taken for the serum to be released and expelled out from the gels.

Lastly, it is possible to identify that the salt release has a trend which is quite similar with riboflavin release through the gellan gels. This can be attributed to the absence of any interaction or binding ability of riboflavin to low acyl gellan gel. It is easy for riboflavin to be released from gellan gels as compared to salt. It is therefore assumed that a weak interaction between riboflavin and gellan will cause a quicker and greater release of riboflavin through the gellan gels as compared to salt.

Chapter 7. Conclusion and Future Work

The thesis has been focused on research experiments which endeavour to explain the reasons affecting the mechanical properties and microstructure of the low acyl gellan gum gels. In addition, it is also concentrated on the linkage between the mechanical properties and microstructure of the gellan gels to comprehend the factors affecting the release from gellan gels. Both the microstructure and the mechanical properties have assisted to understand the release of salt and riboflavin from the gellan gels. The framework of the research study was to comprehend the interaction between the mechanical properties and microstructure of the gellan gels to highlight the impact of the above mentioned factors on the release profile from low acyl gellan gum gels. The same has been performed to enhance the texture and microstructure of the gellan gels which are supposed to manage the release profile from the gels. Section 7.1 includes the conclusion of this study whereas the suggestion for prospective future work on similar topic is part of Section 7.2.

7.1. Main Conclusion

7.1.1. Effect of the Gellan and Salt Concentrations on the Mechanical Properties

The subject experiment has highlighted on the significant role of gellan and salt concentrations on the mechanical properties of the gellan gels. The mechanical properties like for example true stress, true strain, Young's modulus and energy per unit volume of the gellan gels can be affected by the primary essentials like salt and gellan concentration. For all levels of salt, an increase in the gellan concentration

leads to an increase in the strength of the gellan gels. This is due to the greater concentration of biopolymer which causes greater cross link densities within the gel network and hence it creates firm gels.

All gels possess a critical salt (cation) concentration and above the level of this concentration, gellan gels appear as delicate and soft; below this level can however lead to deformation. There exists a relation between the critical cation concentration of gels and the condition wherein every anionic area within prospective junction zones alongside the gellan polymers are dominated by cations. Therefore, maximum contact is observed inside the gel network. The excess number of cations restrains the formation process of cross-links by compelling repulsive forces between polymer molecules and hence undermines the gel structure.

7.1.2. Effect of the Cyclic Compression on the Mechanical Properties of the Gellan Gels

Subsequent to the cyclic compression of the gellan gels, major quantity of serum (water) was expelled from the gellan structure. As a result, new compositions with distinct mechanical properties are expected. The lose water results in the creation of a denser sample and therefore following the cyclic compression, the gel will only get ruptured if additional true stress and true strain is applied. Nevertheless, at the same point, both Young's modulus and energy per unit volume are reduced. Thus, it can be stated that the brittleness and strength of gels will increase because of cyclic compression.

7.1.3. Effect of the Gellan and Salt Concentrations on the Microstructure

Both gellan gum and salt have a significant influence on the microstructure of the gellan gels as evident from the effect of low acyl gellan gum and salt concentrations on microstructure of the gellan gels. The use of different kinds of gellan gum concentrations consisting of different salt concentrations explained the influence of gellan and salt concentration on the microstructural parameters like total porosity; mean pore size and number of pores.

The outcomes of the experiment illustrated that the gellan gum concentration impacts the gellan gels microstructure. The porosity of the gellan gels tends to reduce and consequently the strength and hardness of the gellan gel increases with the increase in the gellan concentration.

The achieved results indicate that the salt concentration which performed as a cross linking agent majorly impacts the gellan gel microstructure. The conclusions demonstrated that an increase in salt concentration is highlighted by the critical salt concentration point which will cause the porosity value to increase and the gel consequently becomes weaker. Conversely, below critical salt concentration point, the porosity value decreases and the gel as a result becomes stronger as the salt concentration is increased.

7.1.4. Effect of the Cyclic Compression on the Microstructure

While investigating on the impact of cyclic compression on the gellan gels it has been noted that the microstructures of the gellan gels are majorly influenced by cyclic compression. This is because of a substantial quantity of water which was expelled from the gellan gel structure. Following the cyclic compression, the porosity value of

gellan gel increases which makes it stronger on one hand but more brittle on the other.

7.1.5. Effect of the Gellan Concentration on Salt and Riboflavin Release from Gellan Gels

Subsequent to the different number of cyclic compression from gellan gels, it can be deduced from the release of salt and riboflavin that both of these releases are influenced by gellan gum concentration. As per the acquired results, a reduction in the release from the gellan gels is observed due to an increase in gellan concentration. This is because of diminished brittleness of gellan gels. Greatest salt and riboflavin release through the gels is observed for gellan gels which were most brittle.

7.1.6. Effect of the Salt Concentration on Salt and Riboflavin Release from Gellan Gels

The primary conclusion derived from the findings of release of salt and riboflavin was that the salt concentration (as a cross linking agent) majorly affects the release from the gellan gels. Above the critical salt concentration point, a decrease of salt and riboflavin release is observed when the salt concentration is increased. At this point with the increase in salt concentration, the gels become less brittle and therefore the release from the gellan gels is reduced.

7.1.7. Effect of Number of Cycles on the Salt and Riboflavin Release

Subsequent to the cyclic compression, the release of salt and riboflavin from the gellan gels depicted that cyclic compression has a considerable impact on the release from the gellan gels. As per the attained results, an increase in the number of

cycles causes the salt and riboflavin release to increase also because of the prolong time required for serum to expel out of the gellan gels. Furthermore, it can also be seen that the major difference in release is during the case of ten cycles with two minute break in between every cycle and the other number of cycles, because of extended time required for the serum to be release and expelled out from the gellan gels.

7.1.8. Comparison between Salt and Riboflavin Release

The findings indicated similarity in the release of salt and riboflavin from the gellan gels. This can be attributed to the absence of any interaction or binding ability of riboflavin to low acyl gellan gum. It is much easier to release riboflavin from the gellan gels as compared to salt. Also, it is probable that the weak riboflavin-gellan interactions causes' quicker and greater release of riboflavin from the gellan gels as compared to salt.

7.1.9. Limitations and Recommendation

The major limitations were observed in this study are as follows:

The present study used gellan gels to identify the flavour release by using cyclic compression test.

There is only limited examinations into microstructure of formulation of gels were carried out in this study.

There is only limited examinations of flavour release was observed in this study.

Therefore this study recommends the below future work.

7.2. Future Work

This section includes some recommendations for future studies in light of interesting observation and conclusions obtained from the study presented here.

- i. Examination of other kinds of gels and mixed gels for cyclic compression test
- ii. Additional examination into microstructure of other formulation of gels
- iii. Analysis on release from other formulation of gels
- iv. Additional examination of release

7.2.1. Examination of other Kinds of Gels and Mixed Gels for Cyclic Compression Test

In order to examine the impact of cyclic compression on mechanical characteristics of the gels, some other kinds of gels and mix gels can be utilized. Examples of such new gels can be various carbohydrate gels, protein gels or a combination of protein and carbohydrate gels.

7.2.2. Additional Examination into Microstructure of other Formulation of Gels

Additional examination of the microstructure of the gels is certainly possible. Amongst the alternatives, the Cryo-SEM can be used to investigate the microstructure of new kind of gels and then compare it with the gellan gels microstructure. Another way of gaining further insight into the study is to the compare the microstructures of pure gels with mix gels.

7.2.3. Analysis on Release from other Formulation of Gels

The release of salt and riboflavin was executed to examine and comprehend the trend of release from the gellan gels after the cyclic compression. It is possible to obtain a better understanding of the release once the trend of release for other formulation of gels is clear.

7.2.4. Additional Examination of Release

There are several other ways by which the release from the gels can be examined. It would rather be fascinating to examine the release from some other releasable materials having distinct molecular weight, distinct charges and distinct polarity as compared to gellan gels and formulations of other new gels. Furthermore, it would be productive to observe the influence of molecular weight and polarity of releasable material on the trend of release from all types of gels.

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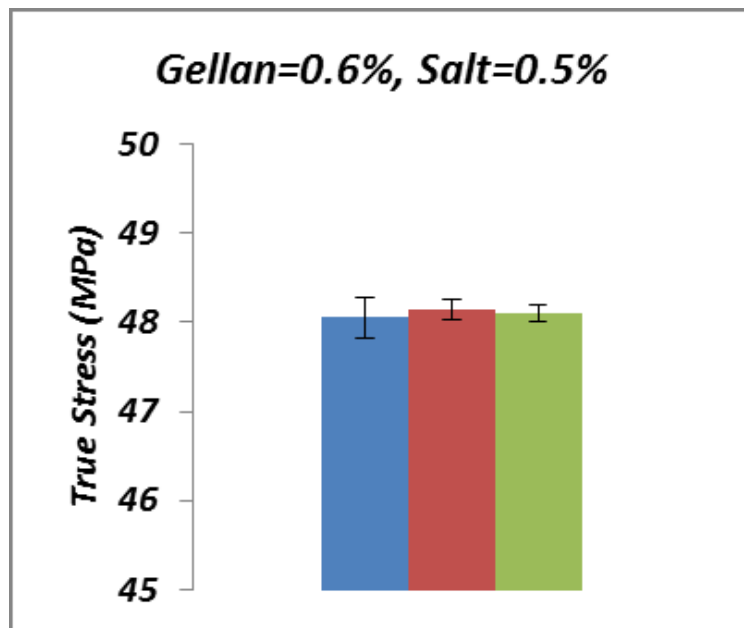
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Appendix

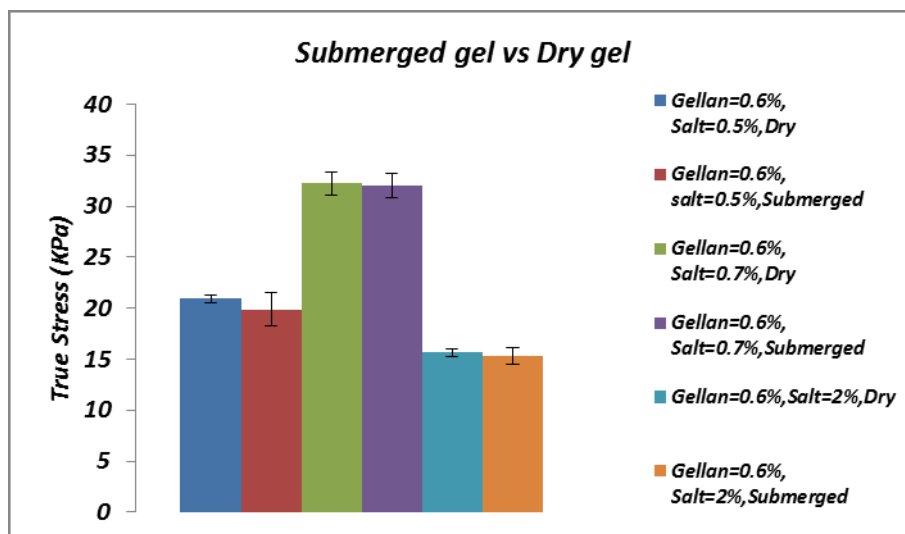
Appendix A

Cross head speed effects on mechanical properties of the gellan gels



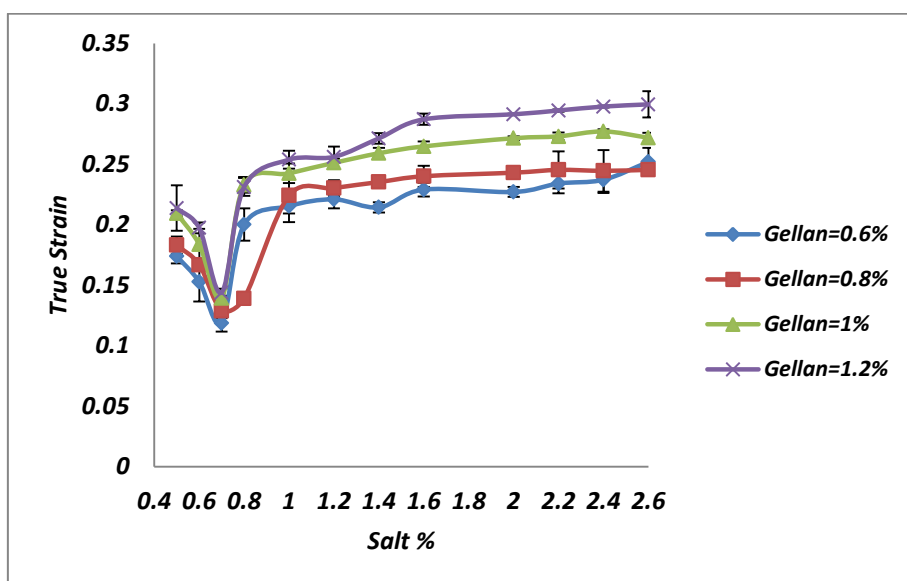
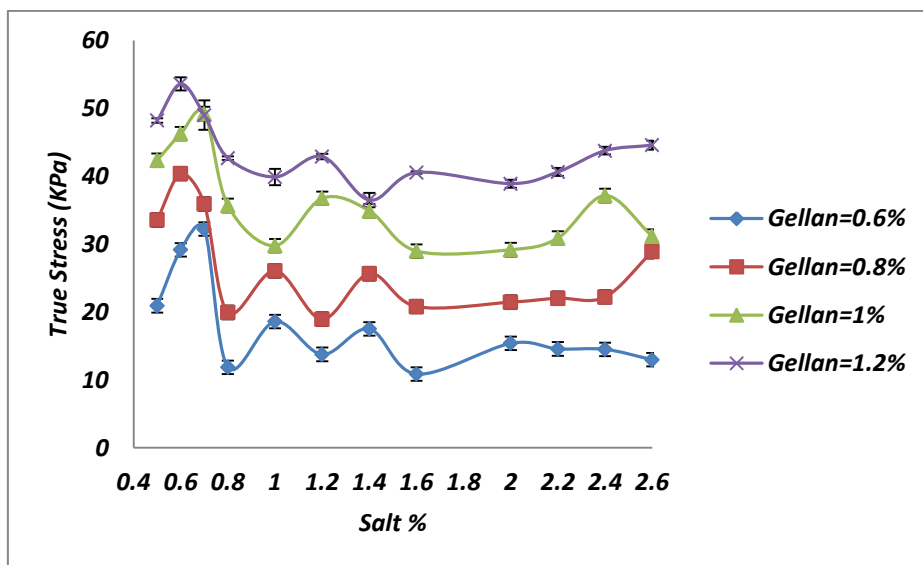
True stress graph of gellan gels at failure point at different cross head speeds. Samples were compressed at 0.5, 1 and 1.5 m/Sec cross head speeds. Graph represent an average of five experiments and error bars show one standard deviation from the mean.

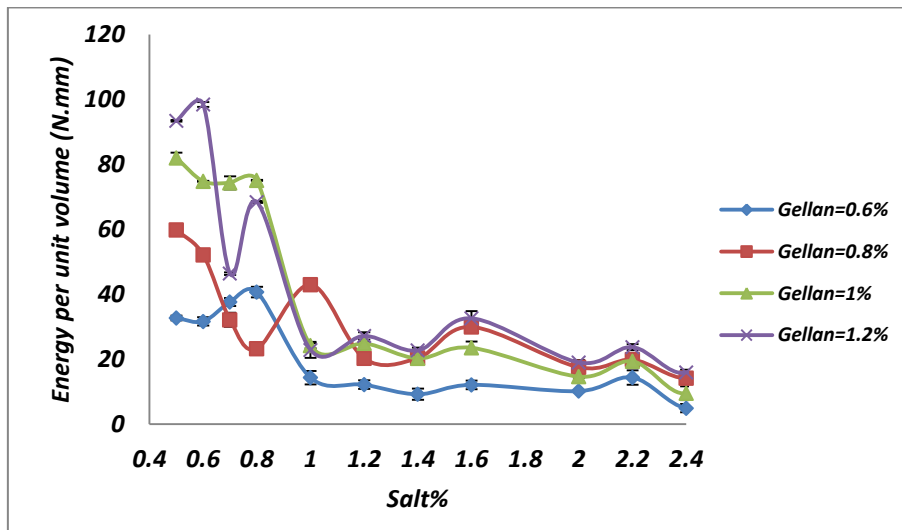
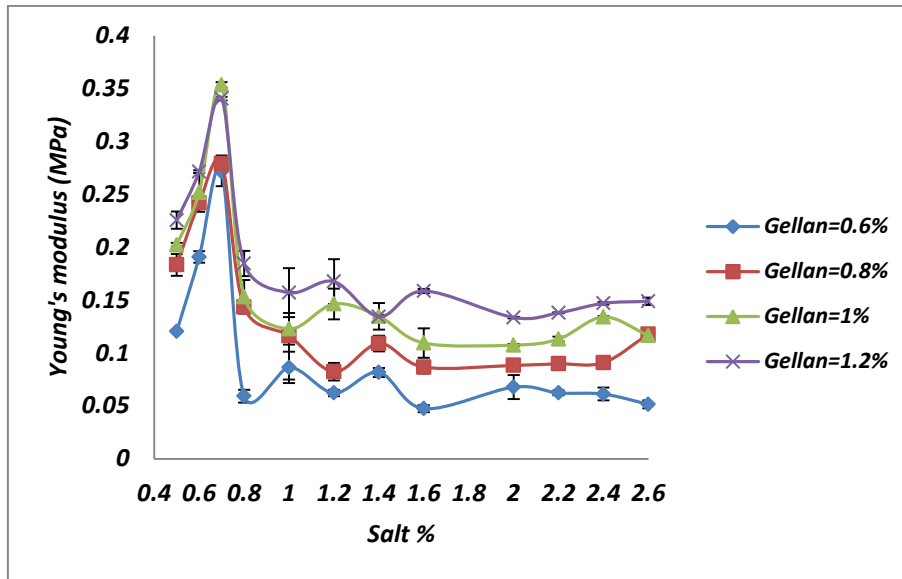
Immersion effects on mechanical properties of the gellan gels



True stress graph of gellan gels at failure of submerged and dry gellan gels. Graph represent an average of five experiments and error bars show one standard deviation from the mean.

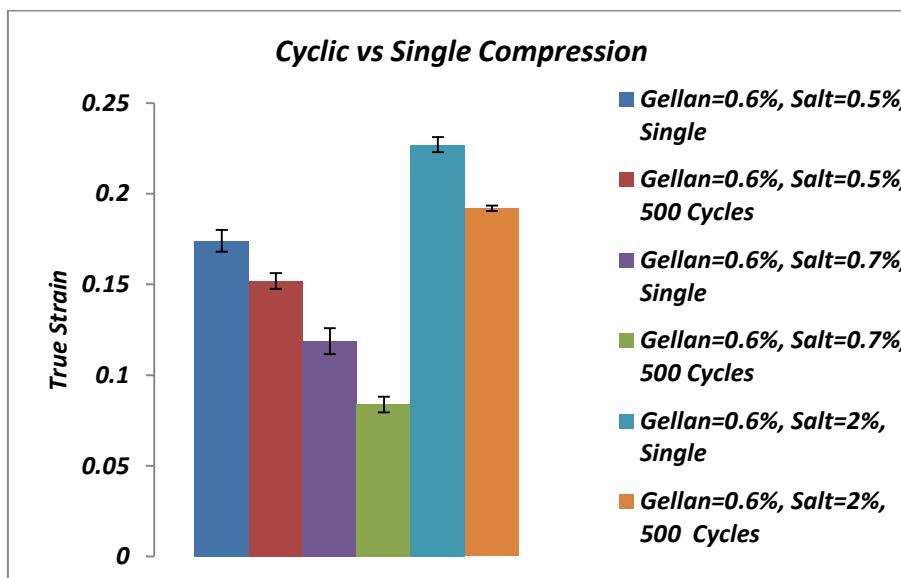
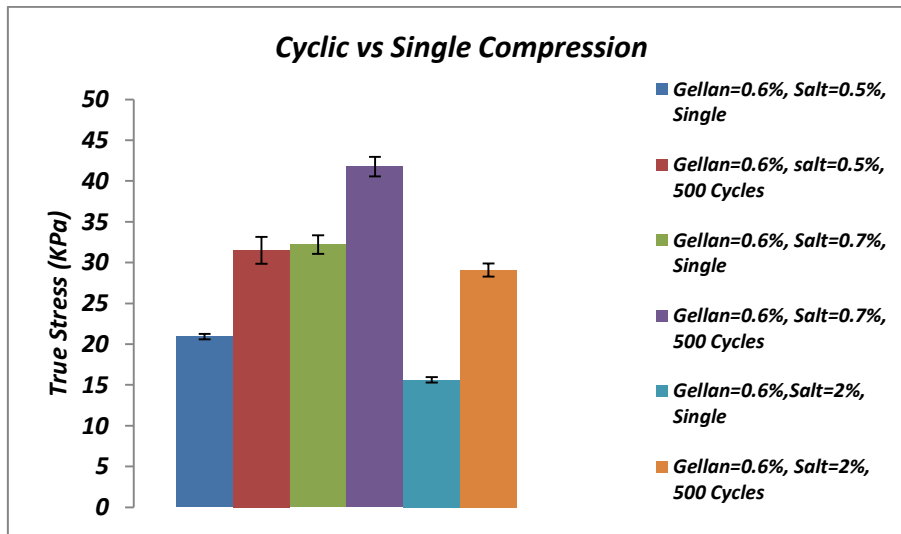
Gellan and salt concentration effects on mechanical properties of gellan gels such as True stress, True strain, Young's modulus and Energy per unit volume)

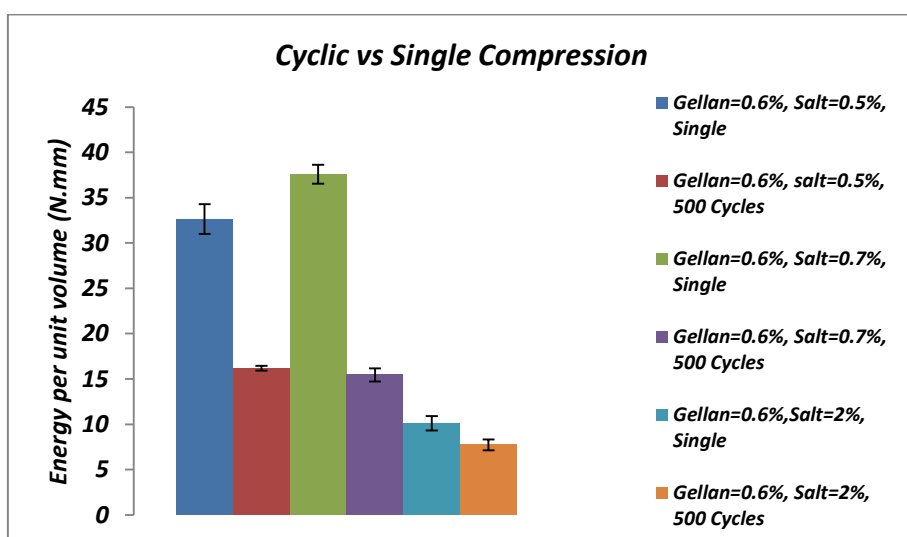
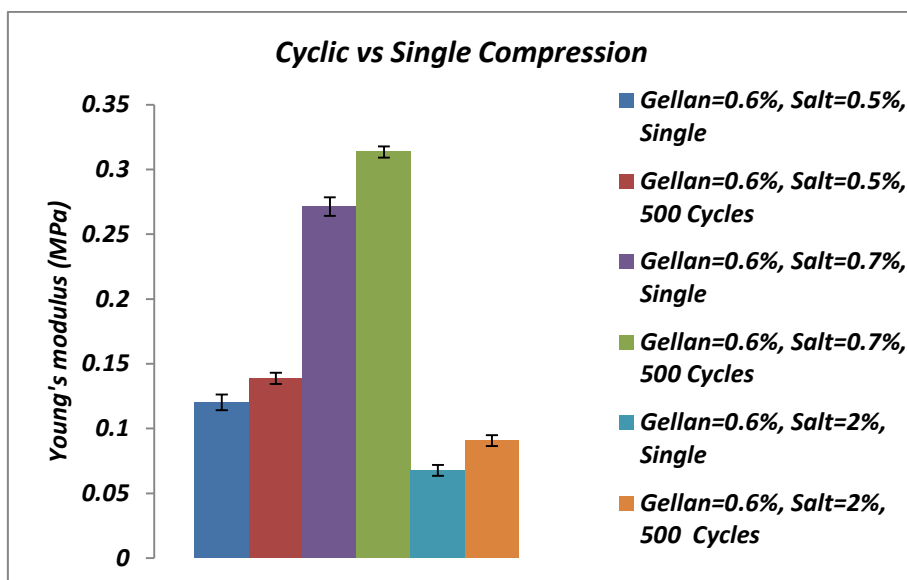




True stress, True strain, Young's modulus and Energy per unit volume graphs of gellan gels at failure point. Graph represent an average of five experiments and error bars show one standard deviation from the mean.

Cyclic compression effect on mechanical properties of gellan gels (a: True stress, b: True strain, c: Young's modulus and d: Energy per unit volume)





True stress, True strain, Young's modulus and energy per unit volume of gellan gels at failure point before and after 500 cyclic compressions. Graph represent an average of five experiments and error bars show one standard deviation from the mean.

Appendix B

Matlab code to determine mechanical properties of gellan gels during cyclic compression (True stress, true strain, Young's modulus and Energy per unit volume).

```
function [] = compeval2()

try

%**** Opening the file ****

%clc ;

close all ;

format short ;

[FileName,PathName] = uigetfile('*.txt','Input cyclic compression data');

if(isequal(FileName,0) || isequal(PathName,0))

    fprintf('Error opening the file, try again later\n');

    return ;

end

FullPath = sprintf('%s\\%s',PathName,FileName);

DataStruc = importdata(FullPath);

if(isfield(DataStruc,'data'))

    data = DataStruc.data ;

else

    data = DataStruc ;

end

%*****

I = [5 10 25 50 100 300 499];
```

```
n = length(I);
res = zeros(n,2);
for i=1:n
    j = find(data(:,4) == I(i));
    k = find(data(j,2) > 0,1); % finding the first positive value
    res(i,1) = I(i);
    res(i,2) = data(j(k),3);
end
disp('Cycle count, Compressive extention (mm)');
disp(res);

catch error
    disp('Error, wrong file selected');
end

end
```

```
function [] = compeval()

try

%**** Opening the file ****

%clc ;

close all ;

format short ;

[FileName,PathName] = uigetfile('*.txt','Input cyclic compression data');

if(isequal(FileName,0) || isequal(PathName,0))

    fprintf('Error opening the file, try again later\n');

    return ;

end

FullPath = sprintf('%s\\%s',PathName,FileName);

DataStruc = importdata(FullPath);

if(isfield(DataStruc,'data'))

    data = DataStruc.data ;

else

    data = DataStruc ;

end

%*****

I = [5 10 25 49];

n = length(I);

res = zeros(n,2);

for i=1:n

    j = find(data(:,4) == I(i));

    k = find(data(j,2) > 0,1); % finding the first positive value

    res(i,1) = I(i);

    res(i,2) = data(j(k),3);

end

disp('Cycle count, Compressive extention (mm)');
```

```
disp(res);
```

```
catch error
```

```
    disp('Error, wrong file selected');
```

```
end
```

```
end
```



```
function [] = mech_rel()
try
%**** Opening the file ****
clc ;
close all ;
format long ;
%*****
[FileName,PathName] = uigetfile('*.txt','Input cyclic compression data');

if(isequal(FileName,0) || isequal(PathName,0))
    fprintf('Error opening the file, try again later\n');
    return ;
end

FullPath = sprintf('%s\\%s',PathName,FileName);
DataStruc = importdata(FullPath);
if(isfield(DataStruc,'data'))
    mech_data = DataStruc.data ;
else
    mech_data = DataStruc ;
end
%*****
[FileName,PathName] = uigetfile('*.txt','Input salt release data');

if(isequal(FileName,0) || isequal(PathName,0))
    fprintf('Error opening the file, try again later\n');
    return ;
end

FullPath = sprintf('%s\\%s',PathName,FileName);
DataStruc = importdata(FullPath);
if(isfield(DataStruc,'data'))
    rel_data = DataStruc.data ;
else
    rel_data = DataStruc ;
end
%*****
I = 0:49;
n = length(I);
A = 346.185;
L = 20;
E = zeros(length(I),1);
Salt = zeros(length(I),1);
for i=1:n
    j = find(mech_data(:,4) == I(i));
    k = find(mech_data(j,2) > 0,1); % finding the first positione
    F = mech_data(j(k:end),2);
    d = mech_data(j(k:end),3);
    e_e = d/L; % engineering strain
    s_e = F/A; % engineering stress
    [~,ind] = max(s_e);
    t = mech_data(ind,5);
    [~,index] = min(abs(rel_data(:,1)-t));
    p = polyfit(e_e(1:ind),s_e(1:ind),1);
    E(i) = p(1);
    Salt(i) = rel_data(index,3);
end
plot(E,Salt,'o');
```

```
catch error
    disp('Error, wrong file selected');
end

end
```

```
function [] = stresseval()
try
%**** Opening the file ****
clc ;
close all ;
format short ;
[FileName,PathName] = uigetfile('*.txt','Input cyclic compression data');

if(isequal(FileName,0) || isequal(PathName,0))
    fprintf('Error opening the file, try again later\n');
    return ;
end

FullPath = sprintf('%s\\%s',PathName,FileName);
DataStruc = importdata(FullPath);
if(isfield(DataStruc,'data'))
    data = DataStruc.data ;
else
    data = DataStruc ;
end
%*****
I = [1 50 100 200 300 400 499]; % index to the cycles of interest
n = length(I);
A0 = 346.185 ;
L = 20 ;
figure;
hold on;
cc = jet(n);
for i=1:n
    j = find(data(:,4) == I(i));
    k = find(data(j,2) > 0); % finding all the positive values
    F = data(j(k),2);
    d0 = data(j(k(1)),3);
    L0 = L - d0 ;
    d_rel = data(j(k),3)-d0;
    L_t = L0 - d_rel ;
    e_t = log(L_t/L0);
    s_t = (F/A0).*(1-d_rel/L0);
    plot(-e_t,s_t,'Color',cc(i,:));
    str = ['Cycle ',num2str(I(i))];
    leg(i,1:length(str)) = str ;
end

xlabel('True strain');
ylabel('True stress [MPa]');
legend(leg,'Location','Best');
title('Effect of cyclic compression');

catch error
    disp('Error, wrong file selected');
end

end
```

```
function [] = workeval()
%**** Opening the file ****
try
%clc ;
close all ;
format short ;
[FileName,PathName] = uigetfile('*.txt','Input cyclic compression data');

if(isequal(FileName,0) || isequal(PathName,0))
    fprintf('Error opening the file, try again later\n');
    return ;
end

FullPath = sprintf('%s\\%s',PathName,FileName);
DataStruc = importdata(FullPath);
if(isfield(DataStruc,'data'))
    data = DataStruc.data ;
else
    data = DataStruc ;
end
%*****
imax = find(data(:,2) == max(data(:,2)),1);
x = data(1:imax,3);      % compressive extension (mm)
y = data(1:imax,2);      % compressive load (N)
res = trapz(x,y); % work done up to max load (N.mm)
%plot(data(:,3),data(:,2),x,y);
disp(['Work done up to maximum load is : ',num2str(res),' (N.mm)']);
catch error
    disp('Error, wrong file selected');
end

end
```

```
function [] = energeval_all()
try
%**** Opening the file ****
clc ;
close all ;
format short ;
[FileName,PathName] = uigetfile('*.txt','Input cyclic compression data');

if(isequal(FileName,0) || isequal(PathName,0))
    fprintf('Error opening the file, try again later\n');
    return ;
end

FullPath = sprintf('%s\\%s',PathName,FileName);
DataStruc = importdata(FullPath);
if(isfield(DataStruc,'data'))
    data = DataStruc.data ;
else
    data = DataStruc ;
end
%*****
I = 0:49;
n = length(I);
res = zeros(n,2);

for i=1:n
    j = find(data(:,4) == I(i));
    k = find(data(j,2) > 0); % finding all the positive value within cycle
    I(i)
    res(i,1) = I(i);
    res(i,2) = trapz(data(j(k),3),data(j(k),2)); % work done up to max load
    (N.mm)
end
disp('Cycle count, Compressive extention (mJ)');
disp(res);
fprintf('The total energy release is %f (mJ)\n',sum(res(:,2)));

catch error
    disp('Error, wrong file selected');
end

end
```